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INTERNAL SUPPRESSORS OF THE h_{III} AND *tu45* MUTANTS OF BACTERIOPHAGE T4

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1. INTRODUCTION

THE h_{III} and *tu45* mutants of phage T4 are a functionally inter-related system within which no simple grouping into independent units or cistrons is possible (Jinks, 1961). Nor can a complementation map be constructed which is co-linear with the order of the genes established by mapping experiments. All the mutants in this system have heat sensitivities which vary between twice and a hundred times that of wild type at 45° C. Heat stable reversions of the mutants, however, are present in all the high titre stocks produced by multiple cycling in host bacteria. The revertants can be selectively isolated and their properties, which are described in this paper, throw additional light on the organisation of the h_{III}-*tu45* segment of the phage genome.

The bacterial and phage strains used and the conditions of assay and crossing are identical with those used previously (Jinks, 1961).

2. RESULTS

(i) *Isolation of reversions of the* h_{III} *mutants*

Reversions of the h_{III} mutants which gave turbid plaques on the mixed indicator (5 parts of S/6: 1 part of KS/4) similar to those produced by wild type phage are present in all high titre stocks of the mutants produced by multiple cycling in host bacteria. Their frequency is sufficiently high (approximately 10^{-3} to 10^{-4}) for direct isolation of the revertants from high density platings. The revertants, however, like the wild types they resemble in plating morphology, aré more heat stable than the h_{III} mutants from which they arise.

They can, therefore, be concentrated by heat inactivation at 45° C. As a preliminary, the sample to be heat inactivated is first recycled at a low multiplicity of approximately ten host bacterial cells per phage particle so as to remove "phenotypic mixing" and hence ensure that the heat sensitivities of the phage particles correspond with their genotype (Jinks, 1961).

In general the recycled stocks were heat inactivated to 10^{-4} survivors, the time required to achieve this at 45° C. being deduced from the inactivation curves. The survivors were plated on the mixed indicator where 1 to 50 per cent. gave turbid plaques, the

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remainder giving the typical clear plaque of an h_{III} mutant. The former were isolated and made into high titre stocks in the usual way. All the h_{III} mutants gave revertants under these conditions. To ensure the independence of the revertants used in the later studies only one was isolated from each recycled sample.

The relatively high frequency of revertants in the high titre stocks of the h_{III} mutants presumably reflects a certain amount of selection for the more heat stable particles during the multiple cycling at 35° C. To obtain more realistic estimates of the reversion rate single cycle stocks made from 4 hour h_{III} plaques were made at low multiplicities. Subsequent heat inactivations gave reversion frequencies of the order of 10^{-6} to 10^{-7}.

(ii) *Isolation of reversions of the tu45 mutants*

Revertants of the *tu45* mutants which are non-turbid on the indicator strain S/6 occur in the high titre stocks of these mutants with frequencies around 10^{-5}. These revertants with their wild type plating morphology also possessed the wild type heat stability and hence could be selectively concentrated in the manner described earlier for revertants of the h_{III} mutants. The frequency of revertants in single cycle stocks of the *tu45* mutants made from 4 hour plaques is around 10^{-7} and always lower than the frequency of h_{III} revertants (see later for detailed comparisons, *e.g.* table 2). The higher frequency of *tu45* revertants in the high titre stocks produced by multiple cycling at 35° C. presumably reflects some selective concentration during this process.

(iii) *Nature of the revertants*

In all, 129 revertants of h_{III} and *tu45* mutants have been crossed to wild type and in every cross the progeny contained a small proportion of the original mutant phenotype, *i.e.* h_{III} or *tu45*. It appears, therefore, that the reversions are due to linked suppressor mutations and not to true back mutations. If this is the case the suppressor mutations should appear in the progeny of the crosses between the revertants and wild type with the same frequency as the original mutant, the two being reciprocal recombinants. No novel phenotype is, however, present in these progenies. This leaves three alternatives for the phenotype of the suppressor mutants (i) they resemble the parents of the crosses which are wild type on the indicator strains used, (2) they are lethal, *i.e.* they do not form plaques on the indicator strains.

That the phenotype of two suppressors of h_{III} mutants is not like that of the h_{III} mutants themselves has been established as follows. The suppressed h_{III} mutants *h41 sug3* and *h41 suo1* (fig. 1) were crossed to wild type and 20 h_{III} recombinants isolated from their progenies. All 20 were backcrossed to a stock of *h41*. All the progenies of these backcrosses were *h41* in phenotype. If the suppressor mutants *sug3*

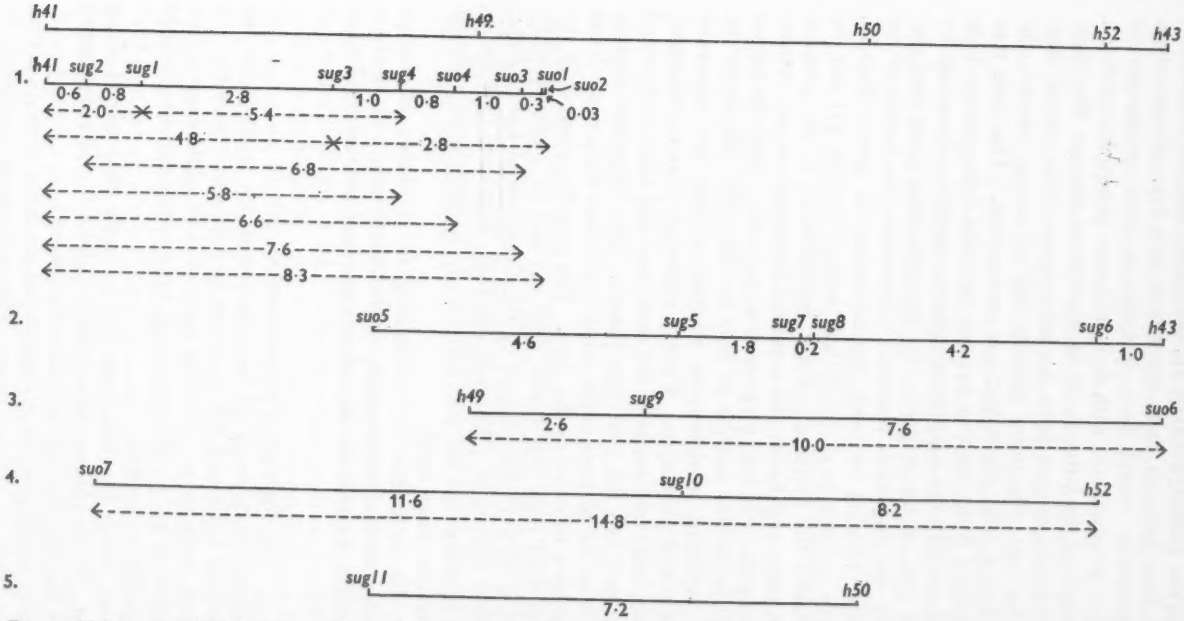

FIG. 1.—Linkage maps 1 to 5 show the positions of the suppressors of the h_{III} mutants mapped relative to the *h* mutant against which they were first isolated and to one another. The extent of the confirmatory evidence of the order of the suppressors revealed by crosses between them is only illustrated in Linkage map 1 for the suppressors of *h41*. The known length of the h_{III} region and the position of the h_{III} mutants within it are shown at the top of the figure for comparison (see Jinks, 1961 for fuller details). As reference to the text shows the relative order of the suppressors has also been confirmed by crosses to *tu45* mutants. Two types of suppressors are indicated, *sug* and *suo* which lead to wild type and non-wild type behaviour on KS/4 respectively.

and *suo1* had h_{III} phenotypes then approximately half the backcrosses would have been between these suppressors and *h41*. Hence, these backcross progenies would have included a small proportion of suppressed *h41* recombinants with their wild type plating morphology.

The absence of the latter in all 20 backcrosses suggests that these suppressor mutants do not have h_{III} phenotypes. For one of these revertants, *h41 suo1*, the more difficult task of testing whether the suppressor had a wild phenotype was undertaken. The wild type plaques obtained from crossing *h41 suo1* by wild type contain both parents and possibly the suppressor mutant, together accounting for some 96 per cent. of the progeny. Of the latter only 4 per cent. are prospectively the suppressor mutant. Ultra-violet irradiation of the parents before crossing increases recombination in phage (Epstein, 1958). Thus 4 minutes of irradiation before crossing *h41 suo1* by wild type raised the frequency of *h41* recombinants in the progeny to 17·3 per cent. and hence the expected frequency of *suo1* recombinants among the wild type progeny to 21 per cent. With this higher expectation the further analysis of only 20 wild type plaques in the progeny is sufficient to determine whether or not the suppressor mutant has a wild phenotype. Twenty wild type plaques, therefore, were isolated, built into stocks in the usual way and each backcrossed to both *h41 suo1* and the wild type parent. The suppressor mutant *suo1* will give no h_{III} plaques in the progeny of either backcross. None of the 20 isolates behaved in this way. Ten gave h_{III} plaques in the backcross to wild type and hence were *h41 suo1* in genotype while the remaining 10 gave h_{III} plaques in the backcross to *h41 suo1* and were therefore wild type. We must conclude, therefore, that *suo1* is neither h_{III} in phenotype nor wild type, in which case it must be lethal on the indicator strains used.

Clearly this technique for establishing the probable phenotype of a suppressor mutant is not readily applicable to large scale analyses and no similar analysis of the other suppressor mutants has been made.

(iv) *Phenotype of the suppressed* h_{III} *mutants*

The plaque morphology of the suppressed h_{III} mutants on the mixed indicator KS/4 and S/6 is similar to wild type and forms the basis of their recognition. On the basis of their plating properties on KS/4 alone at 37° C. the suppressed h_{III} mutants can be classified into two qualitative groups.

(1) Those which like wild type give a plating efficiency of about 10 per cent., all the plaques being turbid "ghosts". The suppressors responsible for this class will be referred to as *sug*.

(2) Those which give no plaques at all on KS/4 even at plating densities as heavy as 10^6 per plate. The suppressors responsible for this class will be referred to as *suo*.

The relative frequencies of these two classes of suppressors obtained

with the various h_{III} mutants are given in table 1. The frequencies of the two classes are those in random samples of 40 revertants isolated by the selective technique described earlier.

The adsorption curve of one of the revertants *h41 sug1* to KS/4 at 35° C. was followed in detail using wild type and *h41* as a control (see Jinks, 1961 for graphs). Under these conditions the total adsorption of the suppressed mutant was less than 5 per cent. and only 0·2 per cent. of the bacterial host cells released viable phage. These figures compare with 8 and 5 per cent. respectively for wild type.

In so far as the revertants are concentrated selectively in an h_{III} stock by heat inactivation at 45° C. they clearly have a heat stability

TABLE 1

The relative frequencies of sug *and* suo *suppressors among revertants of the various* h_{III} *mutants*

Type of suppressor / h_{III} mutant	*h41*	*h49*	*h52*	*h50*	*h43*	Total
sug	16	1	1	1	10	29
suo	8	1	1	0	1	11

comparable with that of wild type. Fourteen of these revertants equally of the *sug* and *suo* types have been compared with wild type for heat stability over a period of four hours of heat inactivation at 45° C. In no case could any difference between the revertants and wild type be detected.

(v) *Phenotype of the suppressed* tu45 *mutants*

All the reverted *tu45* mutants are less turbid on S/6 although some are still sufficiently turbid to be separable from true wild type when jointly plated on S/6. No classification of the revertants on this basis has, however, been attempted. Two types have been recognised on their behaviour on crossing to wild type and on their heat stabilities.

(1) Two of the suppressed *tu45* mutants had heat sensitivities which were identical with those of the *tu45* mutants from which they arose. Furthermore on crossing to wild type both *tu45* recombinants and a novel phenotype occurred with equal frequencies in the progenies. The latter gave plaques which were even clearer than those of wild type on S/6. These were in fact the suppressor mutant. They do not belong to the general system of suppressors under discussion in the paper, therefore they will not be considered further here.

(2) The remaining suppressed *tu45* mutants had the heat stability of wild type. On crossing to wild type no novel phenotype which might have been the suppressor mutant was present in the progeny.

(vi) *Joint suppressors of* h_{III} *and* tu45 *mutants*

Revertants of both the h_{III} and *tu45* mutants are present in the double mutant h_{III} *tu45* stocks with about the same frequency with which they occur in the single mutant stocks and they may be accumulated by selection during heat inactivation. This provides a direct comparison of the reversion rates of* h_{III} *and tu45 mutants. At the same time any double reversions affecting* h_{III} *and tu45 simultaneously will be recognised.*

Twenty independent single cycle stocks of *h43 tu45a* and *h41 tu45a* made from four hour plaques were heat inactivated for five hours at 45° C. and the number of stocks containing h_{III}, *tu45* and joint reversions were scored by plating the survivors on the mixed indicator and on S/6.

TABLE 2

The frequencies of h_{III}, tu45 *and joint reversions in 20 independent stocks of* h41 tu45a *and* h43 tu45a

Type of suppressor / Stock	*h41 tu45a*	*h43 tu45a*	Total
h_{III} *sug*	11	8	19
h_{III} *suo*	6	1	7
tu45	3	4	7
h_{III} and *tu45*	0	1	1

The h_{III} reversions which were recognised on the mixed indicator were tested for their turbidity on S/6. Similarly, the *tu45* reversions which were recognised on S/6 were test for h_{III} characteristics on the mixed indicator. The frequency of the three types of reversions are summarised in table 2. The h_{III} reversions are subdivided into *sug* and *suo* types.

These results confirm the greater frequency of the *sug* type revertants (see table 1) and the higher reversion rate of h_{III} mutants compared with the *tu45* mutants. One joint reversion out of 34 was recovered for *h43* and *tu45a*. Two explanations of the origin of this joint reversion are possible. Firstly, there were simultaneous mutations at a suppressor locus of *h43* and of *tu45a* and secondly, there is a class of suppressors which can suppress both h_{III} and *tu45* mutants. These alternatives are pursued later.

The heat sensitivities of the revertants confirm the classification based on plaque morphology. Thus, those in which only the h_{III} mutant had reverted had the heat sensitivities of the *tu45* mutant while those in which the *tu45a* mutant had reverted had the heat sensitivities of the h_{III} mutant they carried. The joint reversion of *h43* and *tu45a*, on the other hand, had a wild type heat stability.

(vii) *Mapping the suppressors*

In all 21 of the 129 suppressors have been ordered relative to the h_{III} or *tu45* mutants they suppress and also to one another by means of the following crossing programme.

1. All 129 of the revertants were crossed to wild type and in every cross a small proportion of the original h_{III}, or *tu45*, mutant segregated in the progeny—this is our principal evidence that the revertants are indeed due in all cases to suppressor mutations. The probable phenotype of the suppressor mutant has been established in only one case, where it was lethal. In order to determine the recombination frequency between an h_{III} or a *tu45* mutant and its suppressor all the latter mutations have been assumed to be lethal.

In practice the bias introduced by this assumption if incorrect is extremely small unless the suppressor mutant has in fact the phenotype of the mutant it suppresses, in which case the recombination frequencies will be too large by a factor almost of two.

2. Fourteen h_{III} revertants, involving the same h_{III} mutant, were crossed together to determine whether the suppressors lay on the same or opposite sides of the h_{III} mutant they suppress. Thus if they are on the same side the frequency of h_{III} recombinants in the progeny of such crosses will be the difference between the recombination frequencies of the suppressors and the h_{III} mutant. If they are on opposite sides it will be the sum of these frequencies.

3. To determine whether the suppressors of *h41* lay on the *tu45* side of *h41*, revertants of the latter were crossed to *tu45a*. If the suppressor lies between *h41* and *tu45a* then most of the h_{III} progeny will also be turbid; on the other hand if the *h41* locus lies between the suppressor and *tu45a* most of the h_{III} recombinants in the progeny will be non-turbid. The former is true of all the suppressors of *h41* and an example of the segregation observed in such a cross is included in table 3.

4. Six of the *h41* and *h43* revertants were isolated in *h41 tu45a* and *h43 tu45a* stocks and on crossing these to wild type the relative frequency of h_{III} to h_{III} *tu45* segregants in the progenies can be used to establish the order of the h_{III}, *tu45* and suppressor mutations. Thus if the suppressor lies between the h_{III} and *tu45* mutants most of the h_{III} recombinants will be non-turbid while if the h_{III} mutant lies between the other two most of the h_{III} recombinants in the progeny will be turbid. On this test all the suppressors of *h41* fall between it and *tu45a*, while those of *h43* lie on the opposite side to the *tu45* locus. Examples of the segregations observed in some of the crosses are included in table 3.

On the combined basis of these tests the maps shown in fig. 1 have been constructed for the suppressors of the h_{III} mutants.

All the suppressors of the h_{III} mutants map within the h_{III} region delimited by *h41* and *h43*. Furthermore, apart from the possible joint suppressor of *h43* and *tu45a*, all the suppressors of the *tu45* mutants

map on the *tu45* side of the h_{III} region. It is also clear that the two types of suppressors (*sug* and *suo*) of the h_{III} mutants do not map at random to the mutants against which they were isolated. Thus the *sug* suppressors invariably map closer than the *suo* suppressors to the mutant they are suppressing, that is the suppressors which produce the nearest reversion to a wild phenotype map the closest.

If we consider only the suppressors isolated against *h41* and *h43* it appears that *h49* marks the dividing line between the positions of *sug* and *suo* suppressors. Thus, all the *sug* suppressors of *h41* lie between *h41* and *h49*, similarly all the *sug* suppressors of *h43* lie between the latter and *h49*. The same relationship also holds for the two suppressors of *h52*. It breaks down, however, for the suppressors

TABLE 3

The ordering of the h_{III}, tu45 *and suppresssor loci on the relative frequency of turbid and non-turbid* h *recombinants*

Cross	Per cent. recombinants in progeny		Inferred order of loci
	h_{III} *tu45*	h_{III}	
h41 suo1 × *tu45a* . .	3·62	1·32	*h-su-tu*
h43 suo5 tu45a × + . .	4·77	1·18	*su-h-tu*
h43 sug5 tu45a × + . .	3·31	0·21	*su-h-tu*
h43 sug6 tu45a × + . .	0·35	0·12	*su-h-tu*
h43 sug7 tu45a × + . .	2·17	0·64	*su-h-tu*
h41 sug1 tu45a × + . .	0·74	3·12	*h-su-tu*
h41 sug2 tu45a × + . .	0·40	3·00	*h-su-tu*

isolated against *h49* itself, both the *sug* and *suo* suppressors falling on the same side between it and *h43*. Nevertheless, the *sug* suppressor is still the closer of the two.

(viii) *Specificity of the suppressors*

While there is a correlation between the type of suppression and the distance of the suppressor from the h_{III} mutant it is suppressing there is no association between an h_{III} mutant and the suppressors isolated against it. Thus the suppressors of *h41* and *h43* overlap in their distribution along the segment between them as do also the suppressors of *h49*, *h50* and *h52* (fig. 1). It seems unlikely, therefore, that the suppressors will be specific for a particular h_{III} mutant. Apart from one possible joint suppressor, however, there is no overlap in the distribution of h_{III} and *tu45* suppressors. It seems probable, therefore, that the suppressors of h_{III} will not suppress the *tu45* mutants and vice-versa.

Since the suppressors have not been successfully isolated alone, and indeed are probably lethal, all the evidence relating to their

specificity is necessarily indirect. The clearest evidence for non-specificity of a suppressor and the mutant against which it is isolated is obtained where two suppressors isolated against different mutants prove to be allelic in the sense that there is no recombination between them. While a number of probable examples were encountered during the initial screening of the revertants in only one case namely the *sug5* of *h43* and *sug10* of *h52* were sufficient progeny scored to leave no doubt that they were allelic in the sense defined above.

Various double h_{III} mutant stocks were available from earlier experiments (Jinks, 1961). The frequency of revertants in the stocks was compared with that of their component single mutants. From each of the single and double mutant stocks 100 independent single cycle stocks were made from four hour plaques. The reversion rates were then calculated from the observed frequency of stocks containing revertants after first selectively concentrating the latter to a known extent by heating at 45° C. In comparisons of stocks of *h41*, *h49*, *h43*, *h50*, *h41 h49*, *h41 h43* and *h50 h43* no difference in reversion frequency could be detected. The conclusion is, therefore, that the majority of the suppressors which arose in the single mutant stocks must have been capable of suppressing both mutants in the double mutant stocks.

Suppressed stocks of different h_{III} mutants can be crossed together and the frequency of h_{III} recombinants in the progeny determined by plating on the mixed indicator. From the recombination frequency between each h_{III} mutant and its suppressor and between the two h_{III} mutants the expected frequency of h_{III} recombinants in the progeny of such crosses may be estimated assuming specificity and non-specificity of the suppressors. For example, in the cross $h_A\ su_A \times h_B\ su_B$ where the known recombination frequencies a, c and d suggest the following order of the loci and our estimate of b is $d-a-c$,

$$h_A \quad a \quad su_A \quad b \quad su_B \quad c \quad h_B$$
$$\longleftarrow\!\!\!

order of the loci is different from that in the example. However, expectations can be derived in a similar way for any order.

Table 4 contains six illustrations of this test for specificity chosen because they involve *sug* type suppressors which from other evidence are more likely to show some degree of specificity. Since this class of suppressor maps relatively close to the h_{III} mutant it is suppressing the method is not particularly efficient, that is, the two expectations in columns 2 and 3 of table 4 are not always sufficiently different to allow discrimination between the alternatives.

Evidence has already been presented that the two suppressors in the first cross in table 4 are probably allelic. The results in table 4 shows that whether or not they are allelic they are certainly non-specific; each will suppress both *h43* and *h52*. Similarly, the second

TABLE 4

The observed frequency of h_{III} *recombinants in crosses of the type* $h_A su_A \times h_B su_B$ *compared with that of the suppressors are specific and non-specific*

Cross	Observed frequency of h_{III} recombinants	Expected frequency if specific	Expected frequency if non-specific
h43 sug5 × *h52 sug10* . . .	0·0	7·6	0·0
h43 sug5 × *h50 sug11* . . .	1·9±0·2 *	7·1	1·9
h43 sug8 × *h41 suo1* . . .	3·4±0·3 *	7·0	1·8
h43 sug5 × *h49 sug9* . . .	4·6±0·5 *	5·1	3·6
h41 suo1 × *h49 sug9* . . .	3·9±0·3 *	5·8	1·1
h41 sug2 × *h49 suo6* . . .	5·9±0·4 *	5·7	5·3

* In addition to these sampling errors there may be an inherent error, which can be as high as 20 per cent., between recombination values from independent crosses. This must be borne in mind in assessing the outcome of these tests.

cross in table 4 shows that each suppressor will suppress both *h43* and *h50*. The results of the third cross, while not so clear cut, agree better with the expectation based on non-specificity of the suppressors. For the fourth and sixth cross in table 4 the difference between the two expectations is too small to discriminate between them and the observed values agree equally with either. In the fifth cross the two expectations are quite different, the observed value, however, is intermediate and significantly different from both. This anomalous result could have a number of causes. For example, where, as in this case, the recombination frequency between the suppressors is apparently small its estimation as a difference between too larger recombination frequencies is unreliable and could lead to the incorrect ordering of the loci. Unfortunately, no independent ordering of the loci can be made without assumptions about the specificity of the suppressors. On the other hand one of the suppressors may be specific and the other non-specific. The latter possibility can be tested.

Thus if the *sug* suppressor is specific the expected frequency of h_{III} recombinants in the progeny is 5·4 per cent. while if only the *suo* suppressor is specific the expectation is 2·2 per cent. While both are somewhat closer to the observed frequency the latter is still significantly different from both. We can only assume that the alternative explanation of the anomaly is the more likely and hence no conclusion regarding specificity can be made. No evidence in favour of specificity of the suppressors of the h_{III} mutants has, therefore, emerged from any of the indirect tests.

This lack of specificity among the suppressors of the h_{III} mutants contrasts with the specificity they show in their inability to suppress the related *tu45* mutants and the similar inability of suppressors of the *tu45* mutants to affect the h_{III} mutants. Thus in a random sample of 34 suppressors of h_{III} and *tu45* mutants only 1 could suppress the morphology and heat sensitivity of both. It is possible, however, that this apparent single non-specific suppressor is in fact two simultaneous mutations of a specific h_{III} and a specific *tu45* suppressing locus. Observations have been made to distinguish between these alternatives. In mapping experiments the suppressor of the h_{III} mutant (*h43*) is 0·9 per cent. recombination units from *h43* on the side away from *tu45a*. Similarly the suppressor of the *tu45* mutant (*tu45a*) is 6·1 per cent. recombination units away from *tu45a* on the far side of *h43*. Since the distance between *h43* and *tu45a* is 6 per cent. in the same units (Jinks, 1961) the suppressors of *h43* and of *tu45a* map in the same position. Thus if they are independent they must be very closely linked. It is virtually impossible to distinguish between this explanation and one based on a single non-specific suppressor. Two characteristics, however, make an explanation based on a single suppressor more attractive. Firstly, no other suppressors of *tu45* mutants map on the opposite side of *h43*. Secondly, no other suppressors of h_{III} mutants map closer to the *tu45* region. Thus our supposed double suppressor occupies a special position relative to the h_{III} and *tu45* regions, a position one would expect from the relationship between position and function shown by the specific suppressors of the h_{III} and *tu45* mutants.

3. DISCUSSION

The functional relationships of the genes in the h_{III}-*tu45* system can be portrayed in a linear complementation map the essential features of which, apart from its non-correspondence with the linkage map, is that the *tu45* mutants are functionally inseparable from *h49*, *h50* and *h52* but functionally different from *h41* and *h43* (Jinks, 1961). The suppressors show no corresponding groupings in their specificities. Indeed the only consistent specificity among the suppressors is between those which suppress the h_{III} mutants and those which suppress the *tu45* mutants. Thus the suppressors detect a functional difference

between the h_{III} and *tu45* mutants which is not revealed by the complementation tests. On the other hand there appear to be suppressors which do not detect functional differences recognised by the earlier complementation tests. For example, the evidence suggests that most, if not all, the suppressors of *h41* will suppress the other functionally independent h_{III} mutants. A further, though less well established example, is the probable joint suppressor of the functionally independent *h43* and *tu45* mutants.

Although there is no basis for the grouping of the suppressors into functional units or cistrons similar to the complementation test used to classify members of the h_{III}-*tu45* system, they can be grouped into three classes: those which suppress only the h_{III} mutants and map internal to the h_{III} region, those which suppress only the *tu45* mutants and the one suppressor which probably suppresses both. These three classes show no physical overlap on the linkage map; the only mutant which appears to suppress both lying between the other two classes. Thus on this classification there is a correspondence between the spatial and functional relationship of the suppressors.

The class which suppresses the h_{III} mutants has been further subdivided into two types, designated as *sug* and *suo*, on the basis of their plating efficiency on bacterial strain KS/4. There is a complete correspondence between the type of suppressor they lead to and the spatial relations between the suppressor and the h_{III} mutant against which they were both isolated and tested, the *sug* type, in all cases, resulting from the closer suppressors. Although deliberately looked for, but of necessity by indirect tests, no evidence of a different specificity between the two types of suppressors has been found. Indeed, what evidence exists suggests that most, or all, the suppressors of one h_{III} mutant will suppress all others. If this is true then it follows that there is only one type of suppressor of the h_{III} mutants but two types of suppression, the latter depending on the map distance between the suppressor and the h_{III} mutant it is suppressing. For example, if, as seems likely, *sug2* of *h41* (fig. 1) can suppress *h43* and since suppressors of *h43* as distant as *sug2* give a *suo* type of suppression (*e.g. suo5* of *h43*) then *sug2* of *h41* should give a *suo* type of suppression in combination with *h43*. A test of this and similar predictions has yet to be made.

Linked suppressors in microorganisms are rare, the only example in the literature being a single suppressor of leucineless in *Salmonella typhimurium* (Smith-Keary, 1960). "Internal suppressors" of one mutant in the r_{II} system have, however, been found in phage T4 (Feynman, personal communication). Unlinked suppressors are somewhat more common possibly because of the relative ease with which they can be distinguished from true back mutations (see Smith-Keary for review). It is, therefore, remarkable that not only has no example of a true back mutation been picked up in the h_{III}-*tu45* system but that only three unlinked suppressors have

been recovered for the h_{III} mutants, that is, some 2 per cent. of the total.

This study of the suppressors of the h_{III}-*tu45* system has clearly not simplified the rather complex picture which emerged from the earlier complementation tests. On the contrary, the specificities of the suppressors lead to subdivisions of the members of this system into different classes from those delineated by the earlier test. However, perhaps it is naive to expect that a classification of genetic material based on functional relationships should be independent of the functional test applied.

4. SUMMARY

All of the h_{III} and functionally related *tu45* mutants are inactivated at 45° C. with rates ranging from twice to a hundred times that of wild type. Heat stable reversions have been obtained from all of those mutants with frequencies from 10^{-6} to 10^{-7} by strongly inactivating stocks of the mutants.

Of 129 independent reversions examined all are due to suppressor mutations rather than back-mutations. None of the suppressors have been isolated alone. In two cases it has been determined that the suppressors do not have an h_{III} phenotype, while in one of these cases the suppressor has been shown to be lethal. All are closely linked.

The suppressors may be classified as follows.

1. Those which suppress only h_{III} mutants and map within the h_{III} region.
2. Those which suppress only *tu45* mutants and map outside the h_{III} region at the *tu45* end.
3. One example which suppresses both h_{III} and *tu45* mutants and maps between the other two classes.

These three classes thus show a correspondence between spatial relationships and specificity.

The first class may be subdivided into two. Those designated *sug* which plate on KS/4 with the same efficiency as wild type and those designated *suo* which are less leaky on KS/4 than wild type. The former map closer to the h_{III} mutant against which they were isolated than do the *suo* type. There is no evidence of differences in specificity between these two types, most or all appear to suppress all h_{III} mutants.

The functional relationships within the h_{III}-*tu45* system previously established by complementation tests are re-examined in the light of the specificities of the suppressors.

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I am indebted to Drs R. S. Edgar, R. H. Epstein and Mr C. M. Steinberg for much helpful discussion and advice. This work was financed in part by the National Foundation.

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TEACHING GENETICS WITH AN ELECTRONIC COMPUTER

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1. INTRODUCTION

MATERIAL for practical classes in genetics has always presented a difficult problem, which is aggravated by the fact that the best times of the year for practical plant genetics are occupied by vacations or examinations. Cytology can be dealt with fairly easily; suitable material can usually be provided all the year round or can be fixed and kept until required, and the quantities needed are small.

The biggest difficulty in planning genetical work arises from the time that must elapse between making the crosses and scoring the progeny, and this becomes much worse when, as in plant genetics, the experimental material cannot be made to conform to a university timetable. Animals provide better opportunities, though the use of vertebrates such as mice depends on having animal house facilities. By far the best and most widely used organism is *Drosophila melanogaster*, but even this has its limitations, and its extensive use in genetics courses may prove wearisome to all but the keenest students. Nor can it always adequately illustrate the points that one might wish.

Recourse may be had to numerical examples (*e.g.* published results of other people's experiments), to concoctions of loose peas or maize grains, or to entire maize cobs. The first are unconvincing, as being selected to give the right answer, the second are obvious fakes, and maize cobs which have been counted by many generations of students are rather dull. Nor do any of these allow the students either to make or even witness the crosses. By far the most effective data, because they hold the interest of the students, are those from experiments in which they themselves have taken part and whose results are not predetermined—the possibility of the unexpected is always attractive.

Evolution experiments, by their nature, present the greatest difficulties. They may be carried on over a number of years by successive classes, or by using *Drosophila* within the compass of a university course. The latter, however, involves either too much scoring for the class, or too much for the teacher.

Attempts have been made to produce mechanical models of evolutionary systems, but the analogies are far from perfect, and time remains a drastically limiting factor.

Some of the difficulties can be overcome where the course is a specialist one in genetics, and more time and better facilities may be available. In writing this paper, I have particularly in mind the

situation where genetics is being taught as part of a course in botany or zoology.

In February 1960 the final honours classes in botany and zoology at Durham were introduced to a new " organism ". As Fraser (1957) pointed out, the form of the number in an electronic computer allows rather precise analogies with genetic systems. Genetic models may be formed of electronic organisms on which can be impressed whatever rules of genetics one chooses; great flexibility of the system allows the exemplification of a much wider range of genetic phenomena than could easily be achieved with more orthodox material, and interfering side-effects can be eliminated or deliberately included as desired. The superimposing of quasi-random variation brings the systems much closer to reality, and eliminates any question of the predetermination of results.

One thinks primarily of the electronic computer as a very fast calculating machine; but its ability is by no means restricted to calculation. The complex arithmetic which it can perform requires a great deal of administration, and it is the administrative activities of the machine which form the basis of its use in genetic simulation.

To use the machine purely to perform computations from genetical or evolutionary equations would be pointless, and it must be emphasised that the experiments described in this paper are in no sense based on such calculations. Instead, pseudo-organisms are created within the computer, and provided with genotypes which can have phenotypic expression. They are allowed to reproduce according to such laws of chance and genetics as may be desired. They are admirable subjects for experiment.

2. PRINCIPLES OF GENETIC SIMULATION

In the accounts which follow, the aim has been to explain the basis of the electronic analogies which have been used, so that the reader may judge their validity for himself; no special account of computing techniques is given.

The experiments were carried out with a Ferranti " Pegasus " computer, but the general principles have a wider applicability. The working units (" words ") of this computer are linear strings of 38 digits each of which may take one of two values, 0 or 1. Ordinarily, this string of digits is used in one of two ways—either as a simple code giving instructions (" orders ") to the machine, or as a number on a binary scale. But it can in fact be used for any purpose for which a string of digits is suitable, and such a string can be manipulated in several ways—as freight trains may be broken down and reorganised in a marshalling yard. Several words may be shuffled, and their contents recombined. Single digits or groups of digits may be treated in isolation, and may be used to determine activities of the machine; such activities may be regarded as phenotypic expressions of the digits.

In short, the word of the computer provides us with a simple but excellent analogy with a genetic system. The two forms, 0 or 1, of each digit may be considered as two alleles of a gene; these may be given phenotypic expression by causing the precise activity of the machine to depend on the value of the digit at a particular locus in the word. For example, the machine could be instructed to print " round " if the digit is 0, or " wrinkled " if it is 1. Among other things, we may introduce analogies of complementary gene action, additive effects, inhibition, and incomplete penetrance, by making the phenotypic expression of a particular digit depend on the value of the digits (*i.e.* the allelic state) at other loci, or by allowing it to be affected by chance.

One important principle is that the results of computer experiments must be no more predictable than those from experiments with living organisms; there must be no question of predetermination of results by the teacher. The appropriate element of unpredictability may be introduced by the use of sequences of pseudo-random numbers calculated by the machine. Such sequences can never, of course, be truly random, but by the use of suitable generating equations it is possible to produce sequences which in their important properties closely simulate randomness, and in practice may be safely treated as though they were truly random. For convenience, the term " random " will be used throughout this paper for these pseudo-random processes. The sequence used in the programmes to be described is the same as that of an earlier paper (Crosby, 1960), and is a cycle of over two thousand million integers; the cycle may be started at any of its integers, and by allowing the class to choose the starting number the possibility of any predetermination by the teacher is ruled out. Indeed, unless a record of the starting number is kept, the results of any experiment cannot be deliberately repeated.

3. MEIOSIS AND CROSSING-OVER

In the simplest analogy, the word may be considered as a single chromosome or chromatid with a centromere and 37 gene loci, each gene having two possible alleles. The versatility of the computer allows us to do many things with such a chromosome, some of them closely analogous to natural processes.

Fig. 1 shows four such words arranged as two pairs; we are going to consider these as two homologous (but allelically different) chromosomes during meiosis, at the transition from pachytene to diplotene; it is supposed that the chromosomes have divided into chromatids but chiasmata have not yet formed. One digit is the centromere and is printed as an asterisk; teleprinter limitations require this to appear divided too, but this limitation is readily appreciated by the class and causes no confusion.

Now it is quite a simple matter to make the computer exchange sections of words; for example, it could be instructed to transpose the

twelve left-hand digits of the two central words; this would represent chiasma formation with, of course, genetical crossing-over. We could then regard the four words as the four daughter chromosomes of anaphase II. If we had wished, we could have allowed the possibility of more than one chiasma forming at diplotene.

In fig. 2, the four words represent the daughter chromosomes at anaphase II after two chiasmata. Numbering from the top and from

FIG. 1.—Four words of the computer, representing paired homologous chromosomes each divided into two chromatids, at the transition from pachytene to diplotene but before chiasma formation.

the left, one chiasma involved chromatids 1 and 4 between the 9th and 10th digits, and the other chromatids 2 and 4 between the 20th and 21st digits (the reader may disagree with this interpretation; I fell into a similar trap when programming this experiment).

This may be demonstrated to the class on a cathode-ray monitoring tube, and printed out by the machine. Fig. 2 is a facsimile of such a print-out, which suggests a useful exercise for the class. Students are

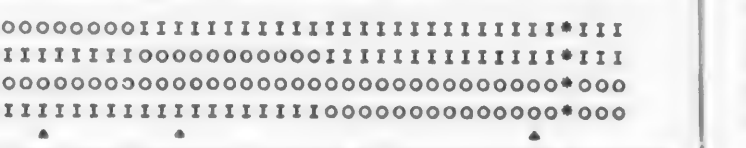

FIG. 2.—Four words of the computer, representing four homologous daughter chromosomes at anaphase II, after two chiasmata.

required to make diplotene diagrams corresponding to print-outs of anaphase II, and find that it isn't always as obvious as it looks; this has the merit of making them think carefully about the logical consequences of chiasma formation.

Chiasma position must not be determined by the teacher; it is one of the variables of the experiment and therefore unpredictable. The simplest way of applying randomness to chiasma formation would be as follows. There are 37 possible positions in which a chiasma may form, and one of these may be chosen by generating a random number in the range 1 to 37; two non-sister chromatids would then be chosen at random and their genes (digits) distal to the chosen position transposed. A second chiasma could be formed in the same way; the two chiasmata cannot be formed simultaneously, and it is essential that the more distal one be done first.

However, in this simple form the process would not give a very realistic analogy. Chiasma formation is not random; it is well known

that as a general rule chiasmata tend to occur less frequently near the centromere and the ends of the chromosomes. What has in fact been done is to take account of end and centromere interference by arranging that the randomisation process will most readily pick positions away from ends and centromere, and least readily close to them. This is done by storing with different frequencies the numbers 1 to 37 in 512 locations in the computer, and choosing one of the locations at random. The frequencies of the numbers follow roughly a flattened normal distribution curve, and the numbers representing the more central positions are therefore the most frequent; thus the chiasma position will most commonly be in the central region of the chromosome.

Chiasma interference has also been taken into account, in the following way. Initially, two chiasma positions are chosen for each meiosis. If they coincide or are adjacent, only the first one chosen is allowed to stand and there will only be a single chiasma. If they are well separated, both are allowed to stand and we have double chiasmata. When the two positions are fairly close together, a random process is introduced which increases the chance of the second chiasma being allowed to stand the farther it is from the first. By the way in which this has been done, chiasma interference is greatest near the ends and centromere.

Obviously, any other pattern of chiasma localisation could have been chosen, and any degree of chiasma interference. The choice was quite arbitrary, and was designed to give the most suitable results for teaching purposes. It would have been easy to introduce chromatid interference, but this has not been done; nor have more than two chiasmata been allowed.

4. LINKAGE AND CHROMOSOME MAPPING

The visual demonstration of chiasma formation in the monitoring tube makes an excellent introduction for the class, and leads naturally into the experiment which is developed from it. This is the construction of a chromosome map from a series of 3-point linkage tests scored by the students themselves.

Eleven of the digits on the chromosome we have been considering have been chosen as gene loci in this experiment. Each gene is named in the orthodox manner, and its phenotype is a symbol which is printed as a single character. For each gene, 1 is taken to be the recessive allele and causes the appropriate character to be printed; 0 is the normal dominant and always causes + to be printed. The class has no prior knowledge of the positions or order of the genes on the chromosome.

Each test-cross is performed, with complete coupling, as follows. Meiosis proceeds (from fig. 1) to anaphase II. One of the four daughter chromosomes is then chosen at random to be one of the

chromosomes in a single test-cross offspring; the homologous chromosome from the testing parent is of course always triple recessive. Thus, as in any test-cross, the phenotype of the offspring is the same as the genotype of the chosen chromosome; the characters of the phenotype are then printed from the chosen chromosome for each of the three loci under test—the appropriate symbol if the digit is the recessive 1, and + if it is 0. The order of printing is not necessarily that in which the genes occur on the chromosome, since this is one of the things which have to be determined by the student; the order adopted is alphabetical according to the gene names. As an example of this process, we may consider fig. 2 and a test-cross with the genes star *, dash —, and tipsy /. From the left, these are in digit positions 4, 12 and 33 respectively, indicated by dots under the diagram. Had the bottom chromosome of this figure been chosen, the offspring would have appeared as — * + since on that chromosome the digits involved are 1, 1 and 0 respectively.

This process may be repeated from the position of fig. 1 as often as required, using successive (and therefore different) numbers from the randomisation sequence. In this way, we can produce a family of as many individuals as desired; a convenient size is 200, and this takes about 1½ minutes to produce. One student is given the print-out sheet of one family and asked to score it, and to determine the order of the genes and the percentages of recombination between them. Fig. 3 is a facsimile of such a print-out, and the reader is invited to score it for himself, and draw the appropriate conclusions before looking at the map which follows later. Although the three genes of this family were chosen deliberately for this paper, the family itself is the only one produced for these three, and is therefore not a selected example.

Subsequent test-crosses, with other gene combinations, start from different numbers in the random sequence and thus have quite different patterns of chiasma formation and chromosome selection.

There are several ways in which the full experiment may be carried out. The teacher may predetermine the test-crosses to be done, and allot them to members of the class; this has the advantage that the crosses may be chosen not only to allow mapping, but also to produce good teaching points, such as the shortage of double cross-overs due to chiasma interference, and the underestimation of long distances due to double crossing-over. On the other hand, there is a considerable advantage, from the point of view of class interest, in allowing the students to choose the crosses for themselves; but particularly with a small class their choice may be such that it may not allow unambiguous determination of the gene order. Where the computer is readily accessible and repeated short visits can easily be made, the most realistic method may be used; the students decide on a few exploratory test-crosses, and from the results of these can plan subsequent series of crosses to give an accurate map of the genes.

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FIG. 3.—Facsimile of a family from a 3-point test-cross. The genes involved are *arc curly mimic* with phenotypes (, = respectively. The dominant phenotype in each case is +. The tested parent had +++ on one chromosome and (, = on the other, though not necessarily in that order. The reader is invited to score this family for himself.

Although the Durham University computer is situated 16 miles away in Newcastle, and so is not easily accessible to Durham classes, a method similar to that last described is quite convenient because the classes involved are in any case too large to handle in a single visit to the computer. First, half the class visits the computer, choosing which test-crosses they will perform subject to the limitation that all genes must be covered by their choice. The test-cross families are scored by the students involved, and from the results a provisional map of the eleven genes is constructed by an assembly of the whole class. The second half of the class then decides which are the best test-crosses to complete an accurate map, and allot these among themselves; their trip to the computer leads to a final mapping of the chromosome during a further assembly of the whole class.

The way in which the final map is determined will depend upon the standing of the class and the intentions of the teacher. With an advanced class, refined mathematical methods may be used, for example to allow corrections for interference; indeed, computer results form an excellent basis on which such mathematical techniques may be taught. At Durham, this experiment is performed at a fairly elementary stage, where such a treatment would be inappropriate, and the primary aim is to help the class to understand the general principles of chromosome mapping based on test-crosses; the mapping is straightforward, if somewhat approximate.

When the class has completed the genetical map, they are then shown the " cytological " map, *i.e.* a diagram giving the exact digital positions of the eleven genes. Fig. 4 shows the first genetic chromosome map produced by our second-year class, together with the cytological map for comparison. The resemblance to the familiar *Drosophila* maps is striking, and the essential difference between cytological and genetical maps is clearly brought home to the class. The figure shows the well-known interference effect by which genes in the centre of the chromosome appear to be relatively farther apart than they really are, while those near the ends and the centromere appear much closer together.

The effect of double cross-overs in causing underestimation of map distances was shown in several test-crosses. For example, the following distances were calculated from individual crosses (in each case the third gene of the cross did not lie between the given two): *d—t*, two separate test-crosses gave 47cM and 46·5cM; *b—a*, 36cM. The distances as finally determined in this experiment were 56·6cM and 41·9cM respectively.

Chiasma interference will have been observed by those readers who have dealt with fig. 3; it was also well illustrated by several of the crosses involved in the production of the map in fig. 4. For instance, in one family of 200 there were 36 single cross-overs in one region and 64 in the other, but only 8 double cross-overs instead of the expected 16 ($\chi^2_{[1]} = 7{\cdot}8$).

These of course are points which could be illustrated with *Drosophila*, although a series of test-crosses involving eleven easily scorable linked genes would be beyond the resources of most departments.

There is, however, one instructive experiment which could not be made with living material. A series of meiosis print-outs is obtained

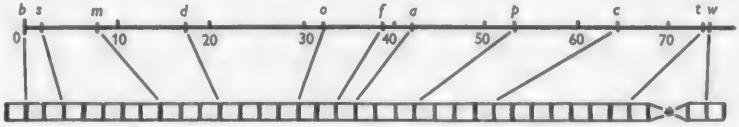

FIG. 4.—Comparison of genetic and cytological maps. The upper map is that determined from a series of test-crosses scored by the class. The lower map is the " cytological " one and shows the actual digital positions of the eleven genes.

as first described. A test-cross is then run, starting from the same number in the random sequence as the print-outs; the test-cross repeats the same sequence of random numbers, and the individuals of the test-cross family therefore correspond, in order, to the previously printed meioses. So when the precise digital positions of the genes

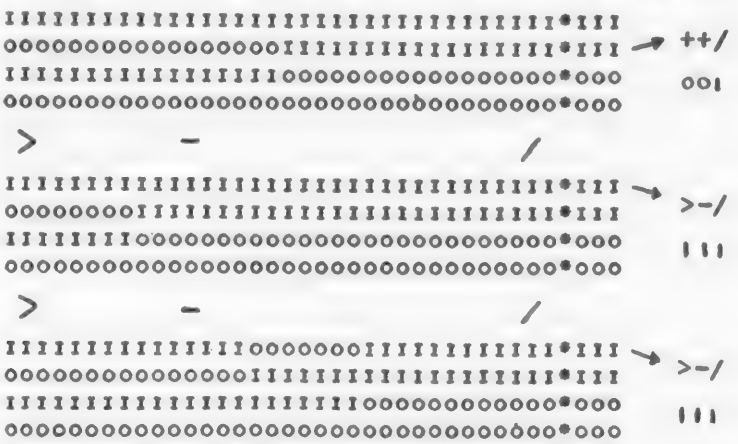

FIG. 5.—Print-outs of anaphase II with the corresponding test-cross progeny shown on the right. For full explanation see text. The digital positions of the genes are indicated ; each phenotype has its digital equivalent shown below it. The lowest example shows a genetically undetectable double crossover.

are known, it is possible for the members of the class to tell which chromosome from each printed set of anaphase II was chosen to produce the corresponding individual in the test-cross family. This is illustrated in fig. 5, which is taken from an actual teaching run. This figure again is a facsimile, the corresponding phenotypes having been excised from the family print-out. The lowest example provides a particularly neat teaching point. It will be seen that the individual

produced was a triple recessive non-crossover; but there is no non-crossover recessive chromosome. Closer examination shows that the chromosome involved must have been the top one, which has a double crossover between *dash* (—) and *tipsy* (/); this provided a good cytological demonstration of a genetically undetectable double crossover between two rather distant genes, and reinforces the point of underestimation of long distances when determined directly. The example comes, in fact, from one of the two families involving *d* and *t* cited above.

The ability to allow examination both of a meiosis and of the offspring derived from it is one advantage which electronic organisms can claim over living ones.

5. POPULATION GENETICS

The possible range of genetic analogy may be greatly extended by using other methods of organising the genetic material within the computer. We are not restricted to the interpretation of a word as a series of linked loci; it could easily be made to represent 38 unlinked genes, or we could consider pairs of digits as allelic pairs of such genes.

Suppose we wish to deal with three unlinked genes; *A*/*a*, *B*/*b* and *C*/*c*. We could represent the genotype of a diploid organism by using six digits in three pairs, each representing one allelic pair. If 0 is the dominant and 1 the recessive, then 100101 would represent *aABbCc* or, more conventionally, *AaBbCc*. Let us consider the production of an F_2 by self-fertilisation of such a triple heterozygote.

Since the genes are unlinked, we do not need to take chiasma formation into account, and our electronic meiosis can be simplified. By deciding on simple adherence to Mendel's second law, it follows that to produce a gamete we have to pick at random one digit from each of the three pairs. Suppose for example that random choice falls on digits 1, 3 and 6 (from the left), that is 101 representing a gamete *aBc*. We store these in the odd positions in a new word which is to represent the offspring, thus, 1-0-1-. A second random choice from the parent, one from each pair as before, gives us the second gamete; fertilisation occurs when these digits are placed in the even positions and we then have the new individual. Thus, if the second choice was digits 1, 4 and 5, the gamete would be 110 (*abC*) and the zygote 110110 (*aaBbcC*). Our electronic organism has reproduced itself once by self-fertilisation.

We may now cause the machine to print the new genotype, or to take account of dominance and print the phenotype, preferably not as gene letters but as symbols representing the characters of the organism, as in the crossing-over experiment. This whole process may be repeated to give as large a family as desired. The print-out is given to the class to be scored, as a family of living organisms would be. It would be easy for the scoring and counting to be done by the

machine, but this would destroy the object of making these experiments as close in form to the real thing as possible.

Different kinds of cross- or self-fertilisation may be simulated in this way, and it is only a short step from controlled crosses to the simulation of breeding populations which may be followed over a series of generations. This gives the possibility of conducting evolutionary experiments. Any desired degree of inbreeding or outbreeding may be imposed on such a population, either in general terms or by detailed simulation of particular kinds of breeding system. Both natural and artificial selection can easily be introduced. Once again, the use of a randomisation technique is fundamental to these experiments.

If one word is taken to represent an organism, many hundreds can be kept within the computer; there is, however, a practical limit to population size, irrespective of the capacity of the machine. In one important respect electronic analogy breaks down—real organisms can reproduce simultaneously, electronic ones can only do so one at a time. This means that if the population size is doubled, the time taken for the population to reproduce itself is doubled. A balance has therefore to be struck between the small populations which would show an excessive Sewall Wright effect, and large populations which would either take too much time for class experiments or would give too few generations. Running time for these programmes is always an important consideration.

In the experiments which are to be described, population size is kept constant during each evolutionary run (though variable between runs), the electronic organisms are hermaphrodite and diploid and behave in the same way as annual plants with no overlapping of generations, and the outbreeding systems are panmictic. These are purely restrictions of convenience, and it would be easy to arrange otherwise if the extra time required by the more complex programmes was relatively unimportant.

When the size and the starting genetic constitution of the population have been decided (that is, the gene frequencies, heterozygosity conditions etc.), the required number of organisms are stored as successive words of the computer, the frequencies of the various genotypes conforming to the required initial conditions. The population is then allowed to reproduce itself as follows.

A parent is chosen at random, and a gamete extracted as previously explained. If the population is panmictic, a second parent is chosen at random and the second gamete extracted from it. The two gametes are combined and the resulting new individual is stored in another part of the computer. This process is repeated until the number of offspring equals the population size, and the whole new generation is transferred to replace the old one. We can now start to produce a further generation from the new population, and so on. It will be noticed that during any generation some organisms will by chance

reproduce several times, some once only, and some not at all, as in any ordinary population.

A simple modification allows for complete inbreeding—only one parent is selected for each reproduction, and provides both gametes. It is easy to introduce any intermediate degree of inbreeding, predetermined as a fraction. When for each offspring one parent has been chosen, this fraction is referred to a random number; this process determines whether the same parent shall be used for the second gamete or a new choice made, the probability of the former event being equal to the inbreeding fraction.

If the experiment is being performed purely as a demonstration, the genetic constitution of the population may be analysed by the computer and printed out as desired. But if it is intended as an experiment for class participation, then it may be arranged to resemble more closely an experiment with living organisms, so that the work done by the students is as far as possible analogous to more orthodox genetical techniques. One example of each of these approaches, as carried out at Durham, will now be given in order to illustrate in a simple way some of the potentialities of evolutionary experiments by computer.

6. THE SEWALL WRIGHT EFFECT

Computer analogy provides an excellent way of showing random fluctuation in gene frequency in small populations. This experiment is more suitable for demonstration than for student participation, which would increase the amount of time required very considerably. One consequence of the fluctuation being random is that the time which is likely to elapse before anything interesting happens is quite unpredictable—a gene may become fixed in a few generations, or be nowhere near fixation after very many; one must be prepared for long runs. In its original form, this experiment involved two unlinked genes; this number has been increased to eight, giving a greater likelihood of at least one gene becoming fixed in a reasonable time. Increase in gene number has not added to the running time—a simple method of gamete selection (Fraser, 1960) can deal with many genes simultaneously.

Population size can be varied from 16 to 512, but the larger sizes are not practicable unless ample time is available; at a size of 64, one generation takes 7 seconds. The population is fully outbreeding, and there is no selection. All genes start with 50 per cent. frequency, and any subsequent fluctuations are due purely to chance. Gene frequency can be followed on a monitoring tube, but this involves interpretation of binary numbers and may be difficult except near zero (extinction of one allele). It is easier for the class to follow gene numbers as they are printed at the completion of each generation. They find this quite exciting, especially if each student is allotted a particular gene, and it brings home to them forcibly the amount of fluctuation in gene frequency that may be caused purely by chance.

To illustrate the kind of results that may be obtained from this experiment, fig. 6 shows the first 120 generations from the first teaching run with the 8-gene programme, in a population of 64 individuals. To minimise confusion, only four of the eight genes are considered in this figure, the choice being governed by considerations of clarity; two of them are seen to have become fixed during the period covered by this figure. Of the other four genes, one became fixed after 63 generations and another at 160. After 199 generations four of the eight genes were far from fixation, although two of them had at one

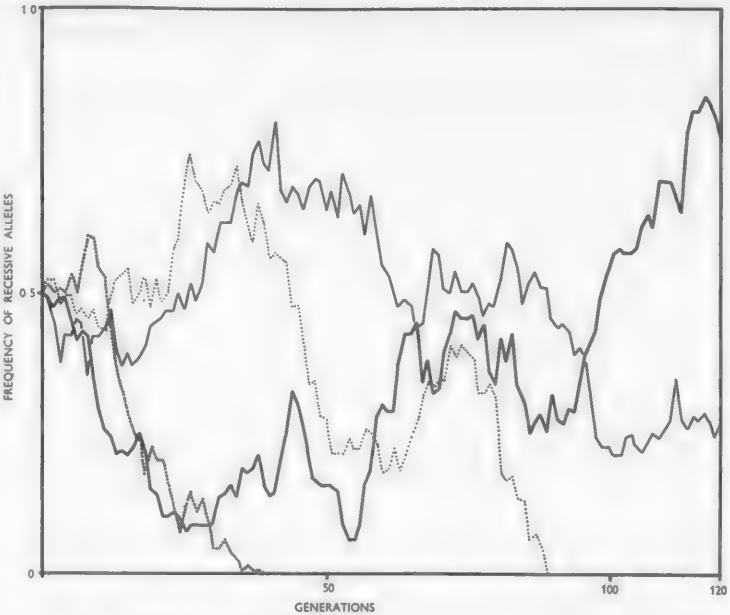

FIG. 6.—The Sewall Wright effect. The frequencies of the recessive alleles of four unlinked genes during the first 120 generations of a population of 64 individuals, with no selection. Changes in gene frequency are due entirely to chance.

time been very close to it. During the same session, a run with a population of size 16 saw seven of the eight genes fixed within 13, 16, 20, 24, 52, 55 and 56 generations respectively.

The class is able to compare populations of different sizes, but the comparisons are themselves affected by chance. The students of subsequent years will be able to get a clearer picture, because the results of their predecessors will be available to them; freak results will be welcome then, when they can be seen in perspective, but in the early stages they are liable to be embarrassing, although they do help to convince the class that the whole thing is not fixed by the teacher.

7. INBREEDING AND OUTBREEDING

This programme is designed to illustrate the important differences between inbreeding and outbreeding in respect of the degree of heterozygosity, the genetic variability of populations, and response to selection. The populations may be panmictic or fully inbreeding, or of any desired intermediate degree of inbreeding.

Unfortunately, in order to give an adequate number of generations in a reasonable time, it has been found necessary to restrict the population size to 256, and the Sewall Wright effect is sometimes quite marked. Even with this size, a generation takes about 50 seconds;

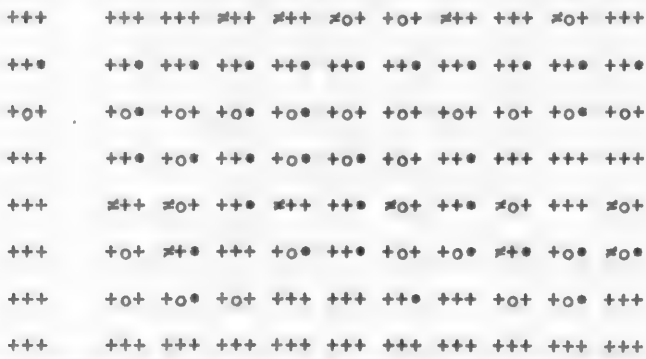

FIG. 7.—Facsimile of some of the test-cross families from the inbreeding-outbreeding experiments after 20 generations of outbreeding. Each line shows the phenotype of the sampled individual (isolated on the left) and those of the ten test-cross progeny derived from it. For example, the top line shows a parental genotype *QqRrSS*.

little improvement is possible with our present computer, except by unrealistic restrictions on the breeding system.

Three unlinked genes are involved, queer (*q*), round (*r*) and star (*s*), with recessive phenotypes ≠ o and * respectively, the normal phenotypes being + in each case. There is no selection against *QQ*, *Qq*, *RR*, *Rr*, *SS* or *Ss* (viability = 1), but there is selection against *qq*, *rr* and *ss*, which singly have viabilities of $\frac{3}{4}$, $\frac{7}{8}$ and $\frac{15}{16}$ respectively. For simplicity, there is no interaction between the genes, and the viability of a multiple recessive homozygote is the product of the appropriate single viabilities. Selection acts immediately after fertilisation, and eliminated individuals are never reckoned as part of the population, which consists entirely of breeding individuals; all surviving organisms have full reproductive capacity.

Selection is subject to chance, and it acts on each new individual in the following way. Each of the three genes is considered in turn. If it is homozygous recessive, then a random number is generated in the range 0-3 for *q*, 0-7 for *r* and 0-15 for *s*. If zero, the individual is immediately discarded and the computer proceeds to form another;

if not zero, the next gene is considered or if the process is now completed for all three genes, the individual goes into the new generation.

At the start of each run, whatever the breeding system, the gene frequencies are 50 per cent., and the genotypes are in precise Hardy-Weinberg proportions. This is an unnatural gene structure, but it allows very good illustration of the essential properties of the different breeding systems; in particular, the rapid increase in homozygosity

TABLE 1

The numbers of different genotypes present in samples of 60 individuals taken after 20 generations from a fully inbreeding population, and after 20 and 60 generations from an outbreeding (panmictic) population.

The possible number of different genotypes is 27. Normal phenotypes have their numbers in bold type. Selection against recessive homozygotes was greatest against *qq*, least against *ss*.

	Inbreeding	Outbreeding	
Generations	20	20	60
Completely homozygous :			
QQRRSS . . .	45	**10**	**22**
QQRRss . . .	10	5	3
QQrrSS . . .	2	1	1
QQrrss . . .	1	...	...
qqRRSS . . .	2	...	...
Heterozygous at 1 locus :			
QQRRSs . . .	...	**5**	**19**
QQRrSS . . .	...	**8**	**3**
QqRRSS . . .	...	4	...
QQRrss . . .	...	1	2
QQrrSs . . .	...	**1**	...
Heterozygous at 2 loci :			
QQRrSs . . .	...	**8**	**5**
QqRRSs . . .	...	**1**	**1**
QqRrSS . . .	...	**1**	1
QqRrss . . .	...	2	...
Heterozygous at all 3 loci :			
QqRrSs . . .	...	**1**	1

on inbreeding is striking. There would be no point in beginning with a population near equilibrium—there would be little to happen.

With complete dominance in a population of living organisms, heterozygotes could not be recognised except by genetical tests. That situation is accepted in this experiment, so that the class may not only see the procedure which would have to be adopted in real life, but may also have an opportunity of participating.

The numbers of recessive homozygotes, which would normally be directly scored in the field, are shown on a monitoring tube, and to this extent the course of evolution can be followed. When it is desired to estimate the gene frequencies, heterozygosity and genetic variability

of the population, a random sample of 60 individuals is taken and test-crossed against *qqrrss*. Each test-cross family is of ten individuals, and the print-out shows the phenotypes of the tested parent and the offspring. Fig. 7 shows a facsimile of part of such a print-out.

The 60 test-cross families are given to the class, who are required to score them and to deduce the genotypes of the sampled individuals. On this basis, the class can form an estimate of the genetic structure of the population. By temporarily sacrificing complete accuracy for speed, preliminary analyses of the population samples can be made

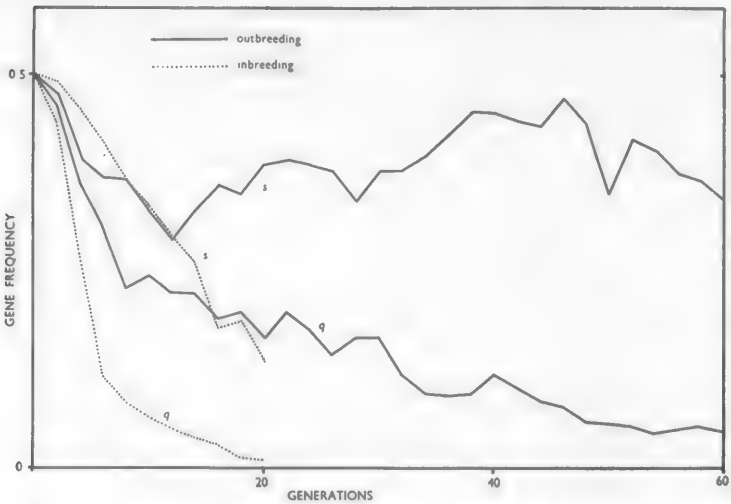

FIG. 8.—The progress of selection in inbreeding and in outbreeding (panmictic) populations, in actual teaching runs. Since all heterozygotes had long since disappeared from the inbreeding population by 20 generations, this run was discontinued then in order to leave more time for the outbreeding runs. Selection against recessive homozygotes only: relative viability of *qq* is $\frac{3}{4}$, of *ss* $\frac{11}{12}$. To avoid congestion, the curves for *r* have been omitted.

as the experiment proceeds. As an example of the kind of picture which may be built up, table 1 shows results from two teaching runs in the same session, one inbreeding and one outbreeding, the former after 20 generations and the latter after 20 and after 60 generations.

It is possible to arrange for the computer to do some population analysis at regular intervals, and to print the results. These are not shown to the class until the experiment is completed, including the treatment of their own results. This further information allows the class to make a more detailed analysis of the progress of evolution, and in particular to see how the gene frequencies change. It also allows comparisons between samples and population. Fig. 8 relates to the same runs as table 1, and shows gene frequencies at intervals of two generations. Among other things, it illustrates rather well

the greater importance of the Sewall Wright effect at lower selection pressures.

The test-crosses provide a statistical exercise as well as a genetical one, in showing variation on an expectation of 5 : 5. The occasional family of ten recessives from a phenotypically dominant parent draws attention to the possibility that the wrong conclusion may occasionally be drawn from a family of ten dominants ; this is why ten is preferred as the family size, rather than a more efficient higher number.

8. DISCUSSION

Although it is much too early for final assessment of the value of this new technique in genetics teaching, my experience so far is encouraging. There is certainly room for improvement, not only in the experiments themselves, but particularly in the method of presentation to the class. The experience of other teachers would be valuable, and I hope that this paper may encourage some of them to try this approach; I will gladly make my programme tapes available to departments which have access to a suitable computer. It is, I think, important that the students should be at the computer when the experiments are in progress. There is an important psychological difference between simply being handed results and actually being present as they are produced.

The experiments have been received with a mixture of curiosity and suspicion. Computers are still a novelty, and the opportunity of seeing one in action is eagerly accepted. There is thus initially a great interest in the experiments. In general, this seems to be maintained, and I think the classes have enjoyed the work and benefited from it. A common attitude to the evolution experiments has been " We've heard all about this in your lectures and read about it in books, but the significance didn't really sink in until we did these experiments and saw how striking the results were ".

Suspicion that the results are faked is very natural, and an important aspect of presentation lies in its removal by making it clear that the teacher has no more control over the results than he has over those from experiments with living organisms. A typical comment is " But you can only get out of the machine what you put in ". An effective counter, quickly appreciated by the class, is " That is equally true of a *Drosophila* culture bottle ". Different results when an experiment is repeated, and comparison with the results of previous classes, also help to convince the students that the experiments are genuine.

Not only do these experiments help to develop the student's imagination, but they provide a good basis on which to discuss the logical relationship between the " laws " of genetics, the experiments on which they are based, and the experiments commonly used to demonstrate them. There is a tendency among students to treat ordinary genetic experiments as " proving " Mendel's laws, and it is not always easy to make them realise that the production of a 3 : 1

ratio in a *Drosophila* experiment does no more than show that *Drosophila* gives the same results as Mendel's peas, and that if any other laws could explain Mendel's results, they would explain the class experiments too.

The quite different logical basis of computer experiments helps considerably in making this point, which is a most important one with honours students, for whom the assimilation of ideas is at least as valuable as learning the rules of genetics. Here, the laws of genetics are known, since they are predetermined in writing the programme; these experiments then demonstrate that given certain predetermined laws, the results tend to follow a particular pattern. For example, the explanation of *Drosophila* linkage results involves the assumption of various interferences in chiasma formation; the computer experiments show that if these interferences really do exist, then the results are very like those from *Drosophila* experiments. This may be emphasised by repeating the computer experiments with the interferences eliminated.

Care must be taken to avoid giving the impression that the computer experiments are valid imitations of *Drosophila* experiments. They do have complete validity, but it is only in respect of the genetics of certain special organisms—electronic ones.

The experiments which have been described are fairly simple ones designed to suit the genetics courses in Durham. They illustrate some of the basic principles of computer simulation, and clearly represent only a small fraction of the possible experiments. But the temptation must be avoided of introducing too much computer genetics, to the neglect of living organisms. Indeed, if computer models of genetic systems become an important part of a genetics course, it is especially necessary that proper use should be made of living organisms, or there will be a danger of the course becoming divorced from reality.

The elaboration of the experiments is another temptation to be guarded against, especially where easy accessibility of the computer removes one of the restraints operating at Durham. It is obvious that the evolution experiments are capable of considerable elaboration, but this is properly a matter for post-graduate work. While there is no doubt that computer techniques have a valuable potential in genetical research, that is beyond the scope of this paper. It is desirable for undergraduate teaching that the experiments should be few, simple, and to the point.

9. SUMMARY

An account is given of some experiments, based on computer simulation of genetic systems, which have been successfully used for practical work in genetics for undergraduate classes in the Durham Colleges.

Accounts are given of visual demonstrations of meiosis, of chromosome mapping by linkage experiments, and of evolutionary experiments demonstrating the Sewall Wright effect and the differences between inbreeding and outbreeding systems under selection.

Acknowledgments.—I would like to thank the Director of the Durham University Computing Laboratory (Dr E. S. Page) for the readiness with which the computer has been made available to us. My thanks are also due to the 1959-60 Final Honours classes in botany and zoology at Durham for their willing and intelligent co-operation during the early trials of these experiments.

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ANEUPLOIDS AND THE CYTOGENETIC STRUCTURE OF WHEAT VARIETAL POPULATIONS

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1. INTRODUCTION

It is well established that *Triticum æstivum* ($2n = 6x = 42$), the common wheat of agriculture, exhibits a low level of meiotic irregularity even in euploid plants of stable and agriculturally acceptable varieties. A small proportion of first metaphase cells in such varieties contains unpaired chromosomes (Thompson and Robertson, 1930; Hollinshead, 1932; Riley, 1960), and the frequency is much higher in heterozygous material (Person, 1956). Clearly irregularities of this nature could lead to the production of aneuploid offspring, and since many aneuploids are viable in wheat such individuals should be found in varietal populations. Hitherto, however, no evidence has been available on the occurrence of aneuploids in stocks of wheat varieties except for the speltoids and compactoids resulting from monosomy or trisomy of chromosome IX. The present paper is therefore concerned with the exploration of the significance of meiotic irregularity on the structure of wheat varieties in terms of chromosome constitutions.

2. MATERIALS AND METHODS

Meiotic behaviour and the frequency of plants with abnormal chromosome constitutions were scored in a number of varieties of the cultivated hexaploid wheat of commerce, *Triticum aestivum* L. emend. Thell. ssp. *vulgare* MacKey. The following varieties were involved, their origins and dates of introduction are given in brackets; Cappelle-Desprez (Hybrid de Joncquois × Vilmorin 27, 1950), Hybrid 46 (Benoist 40 × other hybrids, 1946), Holdfast (Yeoman × White Fife, 1935), Koga II ([Heines Kolben × Garnet] × [Heines Kolben × Raeckes Weissspelziger], 1953) and Atle (Extra Kolben × Saxo, 1937).

Meiosis was scored on preparations made from the spikes of at least 10 tillers collected at random in stocks of each variety. The stocks were those grown in the summer of 1959 at the National Institute of Agricultural Botany, Cambridge, from Foundation seed supplied to the Institute by the breeders of the varieties.

The frequencies of plants with irregular chromosome constitutions were determined from root-tip preparations of seedlings grown from a random sample of the seeds produced by 100 randomly sampled ears harvested from plants grown from the same Foundation seed stocks on which meiosis had been scored. Thus chromosome constitutions were scored on plants one generation removed from those grown from the Foundation seed stocks. It is a pleasure to acknowledge the help of Mr D. B. MacKay, of the National Institute of Agricultural Botany, in providing material for sampling the meiosis and chromosomal regularity of these varietal stocks.

All the plants whose root-tips showed abnormal chromosome constitutions were grown to maturity. Their chromosome constitutions were confirmed principally by meiotic examination but occasionally by checking the chromosome numbers

of a sample of their progenies. Only those plants in which the chromosome constitution was confirmed to be abnormal are so listed in table 5 and are considered in the associated discussion.

In addition meiotic regularity was recorded in the following diploid species related to *T. aestivum*, or involved in its polyploid origins :—

Triticum monococcum L. var. *macedonicum* Papeg. ($2n = 14$).
Triticum boeoticum (Boiss) Scheim. ssp. *aegilopoides* (Link.) Schiem. ($2n = 14$).
Aegilops squarrosa L. ($2n = 14$).
Aegilops speltoides Tausch. var. *ligustica* (Savegna) Coss. ($2n = 14$).
Aegilops longissima Schw. et Musch. ($2n = 14$).

Univalent frequency was scored in the *T. aestivum* varieties on temporary aceto-carmine squashes, and in the diploid species on permanent Feulgen squashes, of pollen mother cells. Initial diagnoses of the chromosome constitutions of plants in the wheat varietal populations were made on temporary Feulgen squashes of root-tips.

3. MEIOTIC BEHAVIOUR

The meiotic regularity of a number of wheat varieties was scored at first metaphase of meiosis (table 1). Frequencies of from 4·2 to

TABLE 1

Meiotic regularity at first metaphase in five varieties of T. aestivum

Variety	Cells	Cells with 2 univalents	Per cent. cells with univalents	Frequency of pairing failure per bivalent
Cappelle-Desprez . .	500	41	8·2	0·0040
Hybrid 46 . . .	500	28	5·6	0·0026
Holdfast . . .	500	21	4·2	0·0020
Koga II . . .	500	34	6·8	0·0032
Atle	500	26	5·2	0·0024
Total . . .	2500	150	6·0	0·0028

8·2 per cent. of pollen mother cells were observed in which one pair of chromosomes had failed to make a bivalent. Since the sizes of the resulting univalents varied in different cells, the irregularities involved several distinct pairs of chromosomes. There was no possibility of determining whether the univalents resulted from asynapsis, that is lack of prophase pairing, or whether they resulted from desynapsis, that is the separation of chromosomes which had paired at prophase without chiasma-formation.

The data for the frequency of cells with univalents in all five varieties are homogeneous ($\chi^2_{(5)} = 8{\cdot}4394$, $P = 0{\cdot}20\text{-}0{\cdot}10$), consequently they may be amalgamated. The frequency of irregular cells based on the combined data is 6·0 per cent., which accords well with previous observations (Hollingshead, 1932 ; Person, 1956; Riley, 1960) and evidently represents a level tolerated by natural selection in this species.

The level of pairing failure in pollen mother cells of *T. aestivum*

may be compared with that in the diploid species from which it has been derived through polyploid evolution. Meiotic regularity was recorded in *Triticum monococcum* and *T. boeoticum*—related to the donor of the A genome, in *Aegilops speltoides* and *Aegilops longissima*—related to the donor of the B genome, and in *Aegilops squarrosa* the D genome donor (table 2). Cells in which one pair of chromosomes were not associated in a bivalent at first metaphase were observed in all these species except *A. squarrosa*. There is a difficulty in testing the homogeneity of the ratios of regular : irregular cells since in the contingency table the expected values for cells with some pairing failure is less than 5. However, as pointed out to us by Professor K. Mather,

TABLE 2

Meiotic regularity at first metaphase of meiosis in five diploid species related to T. aestivum

Species	Cells	Cells with univalents	Per cent. cell with irregular univalents	Frequency of pairing failure per bivalent
T. monococcum . .	50	2	4·0	0·0057
T. boeoticum . .	100	2	2·0	0·0028
A. squarrosa . .	50	0	0·0	0·0000
A. speltoides . .	100	1	1·0	0·0014
A. longissima . .	50	1	2·0	0·0028
Total . .	350	6	1·7	0·0026

this should lead to a spuriously high χ^2 value in such a test. Consequently, since $\chi^2_{(5)} = 2{\cdot}9385$, P = 0·8-0·7, the data for all diploids can be accepted as homogeneous and may therefore be amalgamated.

In the combined data for the diploid species the frequency of irregular cells was 1·7 per cent. which is considerably less than that observed in *T. aestivum*. Indeed there is a significant difference in the ratios of regular : irregular cells in *T. aestivum* and in its diploid ancestors ($\chi^2_{(1)} = 11{\cdot}3982$, P = <0·001).

When, however, the data are expressed as the ratios of pairs as bivalents : pairs as univalents there is no significant difference between the diploids and the hexaploid ($\chi^2_{(1)} = 1{\cdot}5885$, P = 0·30-0·20). The overall frequency of bivalent failure per chromosome pair was 0·0028 in *T. aestivum* and 0·0026 in its diploid ancestors. There was thus little difference in the probability of a pair of chromosomes failing to form a bivalent, the greater frequency of irregular cells in the hexaploid merely resulting from the greater number of chromosomes with pairing properties like those of the diploids.

4. MONOSOMICS AND TRISOMICS

The irregular first metaphase cells which occur with low frequencies in the diploid ancestors of wheat are unlikely to influence population structures since it is improbable that the aneuploid individuals, to

which they might give rise, would be viable at this chromosome level. This is not true, however, of the similar irregularities in *T. aestivum*, in which plants nullisomic, monosomic, trisomic and tetrasomic for each of the 21 chromosomes are known to be viable (Sears, 1954).

Considerable information is available, on the transmission of the univalent chromosomes in wheat monosomics, which can be applied to predict the behaviour of unpaired chromosomes in euploid plants (Morrison, 1953; Sears, 1953, 1954). In monosomics the univalent is transmitted to about 25 per cent. of the gametes and is omitted from about 75 per cent. Functional embryo sacs with 21 and 20 chromosomes have been shown to occur in approximately these frequencies when monosomic individuals are pollinated by euploids.

Morrison (1953) has shown that about 68 per cent. of the pollen grains of monosomics have 20 chromosomes and 30 per cent. have 21 chromosomes. However, as a result of competition between the two classes, 96 per cent. of the pollen which is successful in fertilisation has 21 chromosomes and only four per cent. has 20 chromosomes.

These frequencies of segregation and transmission of the univalent mean that self-pollination of monosomics typically produces about 24 per cent. of 42-chromosome euploids, 73 per cent. of 41-chromosome monosomics and 3 per cent. of 40-chromosome nullisomics. These transmission frequencies were first illustrated by Sears (1953) in the way depicted in table 3.

If the univalents in euploid cells with 20 bivalents and 2 univalents are transmitted independently, and each in the same frequency as the single univalent in monosomics, then 20-, 21- and 22-chromosomes gametes will be formed in the ratio 9 : 6 : 1. Thus, if the frequency of first metaphase cells with two univalents is 0·06 as observed here (table 1), the overall frequency of 20-chromosome gametes will be $\frac{9}{16} \times 0{\cdot}06 = 0{\cdot}0337$. The meiotic observations were based on pollen mother cells, but it is unlikely that any chromosome-deficient pollen grains will succeed in fertilisation in competition with a large excess of euploid grains. Consequently chromosome elimination is only likely to occur as a result of irregular meiosis in megaspore mother cells. If the data for pollen mother cell irregularities are assumed to apply to megaspore mother cells then 20-chromosome egg cells will be produced with a frequency of 0·0337. Assuming that only 21-chromosome pollen functions, 41-chromosome monosomics will arise in the progeny of euploids with the same frequency.

However, the monosomics will reproduce themselves giving frequencies of 0·73 monosomics, 0·24 euploids and 0·03 nullisomics in their progenies (table 3). If these premises are correct, and if there is no differential reproductive capacity of monosomics compared with euploids, a model can be made which describes the frequency of monosomics in populations under various conditions.

Thus if a is the frequency of monosomics in the progeny of euploids, then in a population obtained entirely from euploid parents a would

also be the frequency of monosomics in the population. In the next generation a proportion (r) of the progeny of the original monosomics would again be monosomic, and monosomics would again have newly originated from euploids with a frequency a. Consequently the overall frequency of monosomics would be $a+ra$. Similarly in the third generation the frequency would be $a+ra+r(ra)$. The frequency of monosomics can thus be expressed as a geometrical progression of which the first term is the new production of monosomics from euploids (a) and the common ratio (r) is the proportion of monosomics in the progenies of monosomics. In the conditions described for *T. aestivum*, $a = 0{\cdot}0337$ and $r = 0{\cdot}73$.

TABLE 3

Typical transmission of the monosomic condition in T. aestivum
(after Sears, 1953)

♀ \ ♂	21-chromosome pollen 0·96	20-chromosome pollen 0·04
21-chromosome eggs 0·25	21″ plants 0·24	20″ 1′ plants 0·01
20-chromosome eggs 0·75	20″ 1′ plants 0·72	20″ plants 0·03

euploids (21″) = 0·24
monosomics (20″ 1′) = 0·73
nullisomics (20″) = 0·03

Since the common ratio is less than unity the geometrical progression can be summed to infinity using the formula, $S\infty = \frac{a}{1-r}$. The sum to infinity will be equal to the frequency of monosomics in an old established and stabilised population. In the present case $S\infty = \frac{0{\cdot}0337}{1-0{\cdot}73} = 0{\cdot}1248$, which is the estimated frequency of monosomics.

The expected frequency of trisomics in a wheat population can be similarly calculated. One-sixteenth of the gametes produced from meiotic cells with 20 bivalents and two univalents should have 22 chromosomes, if the segregation is at random. Consequently, if 0·06 is the frequency of irregular meiotic cells the total frequency of 22-chromosome gametes should be $\frac{1}{16} \times 0{\cdot}06 = 0{\cdot}00375$. This is also the frequency with which trisomics should arise from euploids, assuming that only euploid pollen functions due to competition, and that irregular meiosis in megaspore mother cells is at the same rate as in pollen mother cells. Moreover trisomics occur in the progenies of trisomics with a frequency of the order of 0·414, table 4 (Sears, 1954).

In the geometrical progression representing the expected frequency of trisomics, therefore, $a = 0{\cdot}00375$ and $r = 0{\cdot}414$. Consequently, $S\infty = \frac{0{\cdot}00375}{1-0{\cdot}414} = 0{\cdot}00639$, which is the expected frequency in a stable population.

To test the predicted frequencies of aneuploids, the chromosome constitutions were scored of 2027 seedlings of four wheat varieties (table 5). There was a total of 14 monosomics and 3 trisomics, so that the respective frequencies were 0·0069 monosomics and 0·00148 trisomics. Clearly there is a great difference between the expected and the observed frequencies of these two aneuploid classes.

TABLE 4

Typical transmission of the trisomic condition in T. aestivum
(from the data of Sears, 1954)

♀ \ ♂	21-chromosome pollen 0·93	22-chromosome pollen 0·07
21-chromosome eggs 0·60	21″ plants 0·558	20″ 1″′ plants 0·042
22-chromosome eggs 0·40	20″ 1″′ plants 0·372	20″ 1″″ plants 0·028

euploids ($21''$) = 0·558
trisomics ($20''\ 1'''$) = 0·414
tetrasomics ($20''\ 1''''$) = 0·028

However, if the assumptions about the transmission of the univalent chromosomes in euploids and aneuploids are accepted, the formulae for the geometrical progressions can be recalculated to give estimates of a, the new production of aneuploids from euploids in each generation. Thus, dealing with monosomics, the sum to infinity of the series is known as the determined frequency in the population checked, that is 0·0069, and the common ratio, 0·73, is established from the known behaviour of monosomics. Therefore, $S\infty = \frac{a}{1-r}$ can be expressed as $0{\cdot}0069 = \frac{a}{1-0{\cdot}73}$, consequently $a = 0{\cdot}001863$.

Thus, on the observed population frequency of monosomics, 0·1863 per cent. of the progeny of euploids should be monosomic. When the chromosome numbers were checked of 200 plants in the progenies of euploid plants, in the variety Holdfast, one monosomic was found. Although a considerably larger sample would need to be examined to check the predicted production of monosomics, it may well be that the estimate is reasonably correct.

Similarly the production of trisomics from euploids can be estimated

TABLE 5

Chromosome constitutions of samples of varietal populations of T. aestivum

Variety	Plants examined	Euploid (42-chromosomes)	Monosomics (41-chromosomes)	Trisomics (43-chromosomes)	Others	Frequency of aneuploids
Cappelle-Desprez . .	539	537	1	0	1 = 42 with telocentric	0·0037
Hybrid 46 . . .	507	499	4	1	1 = 44 with telocentric 1 = 42 with larger chromosome 1 = 21/42 chimaera *	0·0160
Holdfast . . .	511	505	5	1	...	0·0120
Koga II . . .	470	463	4	1	1 = 44 1 = 44 with 2 telocentrics	0·0150
Total of four varieties .	2027	2004	14	3	5	0·0108
Holdfast euploid parents	200	199	1	...	...	0·0050
Druchamp × Soissonais F_2	59	55	2	1	1 = 42 with telocentric	0·0680

* Not scored as aneuploid.

from the observed frequency of trisomics in the population. This was 0·00148 and again equals the sum to infinity of the geometrical progression. Consequently when $S\infty = \frac{a}{1-r}$ and $r = 0{\cdot}414$, $0{\cdot}00148 = \frac{a}{1-0{\cdot}414}$, and $a = 0{\cdot}000867$.

Thus 0·0867 per cent. of the progeny of euploid wheat plants should be trisomic.

The causes may now be considered of the errors in the estimated occurrences of monosomics and trisomics, in varietal populations, based on the observed frequencies of first metaphase irregularities. It should be indicated at the outset that no information is available to show how closely the populations sampled resembled stabilised populations. Those examined were derived from Foundation seed of established varieties which was produced by the breeders of the varieties. In building the Foundation stocks the breeders may well have exercised selection, against rogues, which would have reduced the proportion of aneuploids.

If, however, as seems probable, this was not solely responsible for the deficiency of aneuploids, three others factors should be considered. These are the assumptions that:—

(*a*) There is no difference in the reproductive efficiency of monosomics and trisomics compared with euploids.
(*b*) The two univalents in euploids segregate at random.
(*c*) The frequency of irregular first metaphase in megaspore mother cells is the same as that in pollen mother cells.

General experience with monosomics and trisomics in wheat suggests that they are usually as efficient in reproduction as euploids. However, even if the fertility of monosomics was reduced, for example, to 90 per cent. of that of euploids, the frequency of monosomics would be $S\infty = \frac{0{\cdot}0337}{1-(0{\cdot}73\times 0{\cdot}90)} = 0{\cdot}0982$. Similarly the frequency with 50 per cent. fertility would be 0·05937. These values, and others similarly calculated for trisomics, are still greatly in excess of the observed frequencies, so that it seems unlikely that differential fertility can be an important factor in causing the discrepancy between the observed and predicted frequencies of aneuploids.

In wheat monosomics the univalent chromosome usually divides into chromatid-halves at first anaphase of meiosis and may then be included or excluded after second anaphase movements. However, chromosomes which have paired at prophase without chiasma-formation sometimes remain secondarily associated as univalents (Person, 1955; Riley and Chapman, 1957). Occasionally the secondarily associated univalents then orientate on the first metaphase plate and separate one to each pole in late anaphase. If the two

homologous univalents in irregular euploid cells often separated in this way, predictions based on the behaviour of univalents in monosomics would be erroneous. However, observation of the irregular cells of euploids suggests that the univalents rarely co-orientate in this way and that the behaviour of the two univalents is generally independent. Consequently non-random segregation of univalents is unlikely to be a source of error in estimating the frequencies of monosomics and trisomics in populations.

The remaining possibility of error lies in the assumption that pairing failure occurs in megaspore mother cells in the same frequency as in pollen mother cells. Meiosis has apparently never been studied in megaspore mother cells in wheat. Indeed it would be extremely difficult to assemble a sample of adequate size to determine the level of meiotic regularity. However, it might be expected that meiotic irregularity would be less common than in pollen mother cells. For each ovary in wheat produces only one embryo sac and one seed, consequently every megaspore meiosis influences fertility and any inefficiency in the meiotic process would immediately diminish the reproductive effectiveness of an organism selected for a high yield of seeds. By contrast a small proportion of cytologically unbalanced-pollen grains would not influence fertility in a self-fertilising plant in which most of the pollen produced is wasted. Therefore the selection pressures acting to produce meiotic regularity are probably more stringent in their impact on megaspore mother cells than on pollen mother cells. Thus a higher level of pairing failure might well be tolerated at meiosis in pollen mother cells. The discrepancy between the frequencies of irregular pollen mother cells and the observed frequencies of aneuploids, which arise from irregular megaspore meiosis, could well be a manifestation of the different courses of division in the two types of cells.

Indeed, using the estimated frequencies of the origin of monosomics and trisomics from euploids, which were derived from the observed occurrences of these aneuploids in populations, further estimates can be made of the frequencies of irregular megaspore mother cells. If the frequency of the production of monosomics from euploids is 0·001863, then the frequency of irregular megaspore mother cells from which they arise must be $0{\cdot}001863 \times \frac{16}{9} = 0{\cdot}003301$. A similar calculation, based on the frequency of trisomics, gives an estimate of $0{\cdot}000914 \times 16 = 0{\cdot}013872$.

Thus a bivalent is estimated to have failed in 0·3301 or in 1·3872 per cent. of megaspore mother cells on the basis of the observed occurrence of monosomics or trisomics respectively. The differences between these estimates may result from differences between the viabilities of trisomics and monosomics, or between the gametes from which they are derived. Alternatively some of the excess of trisomics in the population studied may have resulted from the decay of the tetrasomic condition through segregation. Finally the difference

may merely be due to sampling errors in the observations of aneuploid frequencies in the population studied. Irrespective of the cause of the difference, however, it seems that there must be a marked reduction in the occurrence of irregularities at meiosis in megaspore mother, compared with pollen mother, cells.

5. OTHER ANEUPLOIDS

Monosomics and trisomics can of course give rise by segregation respectively to nullisomics and tetrasomics. Nullisomics in wheat are of low vigour and are usually either completely sterile or very infertile. Consequently nullisomics would probably be quickly eliminated from the population. Indeed any present in the population would probably have been newly derived from a monosomic parent. On the basis of the 2027 seedlings checked (table 5) 0·69 per cent. of the population proved to be monosomic. With this population frequency of monosomics, and assuming that 3 per cent. of the progeny of monosomics are nullisomics (table 3), then 0·013 per cent. of the varietal population should consist of newly arisen nullisomics. None was discovered in the varieties examined but the sample was rather small for the detection of such a minor component of the population.

By contrast with nullisomics, tetrasomics are viable and fertile and are partially stable. Tetrasomics for most chromosomes produce about 80 per cent. tetrasomics in their progenies. Consequently, although they are produced from trisomics with only about the same frequency as nullisomics from monosomics, once they have arisen they will form a persistent component of the population. It is not surprising, therefore, that three tetrasomics were observed amongst the 2027 seedling examined (table 5).

Most of the non-tetrasomic progeny of tetrasomics are trisomic. Thus the tetrasomic component of a varietal population will represent a source of inflation of the proportion of trisomics.

The univalent chromosomes in euploids and monosomics or in those trisomic cells in which no trivalent is formed, may occasionally misdivide at meiosis to give rise to telocentrics or isochromosomes. In the present work no isochromosomes were observed presumably because plants with aberrant chromosome conditions were selected from the examination of somatic chromosomes and isochromosomes would be indistinguishable from a number of normal chromosomes with median centromeres. However three plants were obtained with telocentric chromosomes. Two of these had tetrasomic numbers and one had a euploid number (table 5).

Clearly misdivision of this nature can lead to the inclusion of stable chromosomal off-types in a variety. Thus for example the 42-chromosome plant with one telocentric found in Cappelle-Desprez could segregate to form 42-chromosome offspring disomic for the telocentric condition. Unless selectively eliminated these would then form persistent cytological deviants in the population.

6. CONCLUSIONS

(i) *The population structure of wheat varieties*

From the present work it is apparent that there is a small component of varietal populations of *T. aestivum* continuously moving through aneuploid conditions. Aneuploids arise from the euploid condition and slowly over several generations most of the derivatives of the initial aneuploids revert to euploid. This slow turn-over of aneuploids depends upon the presence of considerable genetic duplication associated with a diploid-like meiotic pattern in which bivalent formation occasionally fails. The bivalent failure may arise from the combination of the chromosome sets of three diploid species which also occasionally form univalents. However, there seems to be little deleterious effect, since most aneuploid conditions do not disturb the fertility or the vigour of wheat. The only components of the population permanently modified arise through the effective fixation of tetrasomy and through the accumulation of misdivision products.

It may be assumed that all chromosomes are equally likely to fail to pair at meiosis in euploids, and to give rise to aneuploidy. However, there is as yet no direct evidence on this, although the differences in plant morphology between some of the aneuploids extracted (plate I) give some confirmation.

The extent of genetic duplication is sufficiently great in *Triticum aestivum* for considerable laxity in the control of chromosome organisation to be tolerated. Indeed it may be that the passage of chromosomes through the monosomic phase promotes the retention of much duplication. Since the least deviation from the euploid phenotype, which is presumably the optimum expression, will occur in those plants efficiently buffered by duplications. Thus selection for masking duplications may occur.

It is noteworthy that the exposure of parts of the genotype to the hemizygous condition is only likely to be tolerated in an inbred, and largely homozygous, organism such as wheat. In an outbreeder monosomics would probably expose deleterious recessive genes and so would reduce the overall efficiency of the population. Consequently selection would operate against a meiotic system which caused the production of aneuploids. Under these conditions it is perhaps surprising that genes which are ineffective when hemizygous, such as the speltoid suppressor (*Q*) on chromosome IX, are not uncommon in wheat (Sears, 1954), since monosomy for a chromosome carrying such a gene must be immediately apparent.

Paradoxically also, the genetic deviation which results from the production of monosomics may also lead to a return to even greater stability. For the euploids derived from monosomics will be completely homozygous in the homologues which have passed through the monosomic phase. In the generations immediately following hybridisation, when the frequency of monosomics is considerably

higher than in stabilised varieties (table 4), this may have some selective advantage in speeding the return to homozygosity. In established varieties passage through the monosomic condition will also remove heterozygosity from chromosomes carrying newly arisen mutants.

Thus the dynamic state of the chromosome composition of wheat populations seems to lead to only minor adverse effects and may indeed confer certain selective advantages. The production of aneuploids and their reversion to euploidy may merely be the fortuitous outcome of the continuation in the polyploid of the bivalent failure which occurs in its diploid ancestors. At the polyploid level however, due to duplications, the individuals with irregular chromosome constitutions, to which the pairing failure inevitably gives rise, are viable.

(ii) *The genetic purity of wheat varieties*

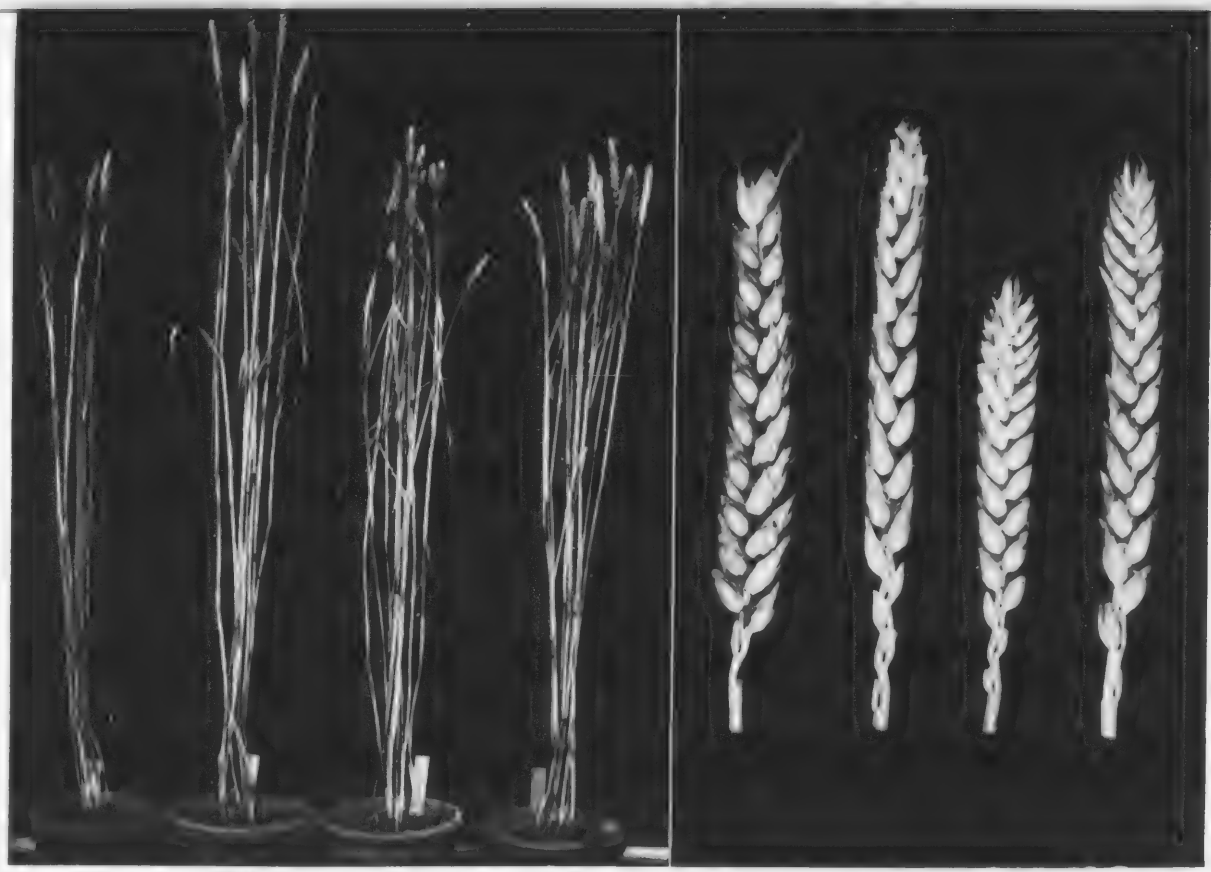
In many countries organisations exist to ensure that pure stocks of seeds of many crops, including wheat, are available to the farmer. There can be no doubt of the great value of this service in ensuring that the varieties distributed are those named, and that stocks are not mixed with the seeds of other varieties. In addition it is obviously important to be sure that there is no undue contamination with seed borne diseases or with the seeds of weed species. However, it is sometimes also the task of such organisations to ascertain that the genetic variation in the crop is minimal, and to this end standards of genetic purity are set.

It has been the purpose of this paper to demonstrate that a fairly high level of chromosomal irregularity is inevitable in the wheat crop. In the varieties examined plants with irregular chromosome constitutions ranged from 0·37 per cent. to 1·60 per cent. of those examined, with a mean of 1·08 per cent. (table 5). (In the oat variety Sun II, 1·10 per cent. of the 631 plants examined were aneuploid.) These are frequencies much greater than those tolerated for morphologically distinguishable " off-types " by some seed certifying organisations. Consequently, in as much as irregular chromosome constitutions may lead to phenotypic deviations from the standard type, it may be that biologically impossible targets are set.

The most common class of irregularity in wheat arises from the occurrence of monosomics. Wheat varieties differ in the phenotypic effect of the monosomic condition. This can be illustrated from the difference between Chinese Spring and Holdfast neither of which, it should be indicated, are now commercial varieties. Whereas in Chinese Spring none of the monosomics, except that for chromosome IX, differs phenotypically from the euploid under favourable conditions, in the variety Holdfast many different monosomics cause distinctive modifications of the phenotype (plate I). This, rather than real differences in genetic behaviour, may be the cause of observations commonly made by agronomists that some wheat varieties are more

Plate

Mature plants, and ripe ears from the same plants, from the sample of the variety Holdfast checked for chromosome constitution, (*a*) and (*e*) 42-chromosome euploid ; (*b*) and (*f*), (*c*) and (*g*), (*d*) and (*h*) 41-chromosome monosomics.



(a) (b) (c) (d) (e) (f) (g) (h)

constant than others. However, it is clear that in a variety with many distinguishable monosomics, the level of genetic impurity permitted should be relaxed compared with some other varieties. It must be recognised that this assumes a high ascertainment of morphological deviants, whereas under field conditions some of the aneuploid deviants would probably fall within the range of environmentally-caused variation of the euploid majority of the crop. Nevertheless the standards of genetic purity demanded should be so devised that they fit a level realisable in the crop.

It has been indicated earlier that the initial aneuploid states may lead to the fixation of tetrasomic or of misdivision conditions, in the latter case as disomic telocentric plants with euploid numbers. The tetrasomics for most chromosomes are recognisably different in morphology from the standard type (Sears, 1954), and most disomic telocentrics deviate from normal in the direction of the corresponding nullisomic. The probability of the accumulation of these aberrant, and inefficient, classes in unselected stocks of a wheat variety must emphasise the importance of the continued return to selected stocks, and must stress the importance to agriculture of supplies of pure seeds. The only conern is that, in attempting to obtain genetically standardised seed supplies, a level of purity should not be demanded which the meiotic system of the plant does not permit.

(iii) *Spontaneous aneuploids and wheat breeding*

Person (1956) has pointed out that the level of meiotic irregularity, particularly pairing failure, is much higher in heterozygous wheat material than in stable, more or less homozygous, varieties. Indeed he has demonstrated that, from the F_1 and through the succeeding backcross generations, the return to the normal level of pairing failure follows the return to homozygosity.

It might be anticipated, therefore, that the production of aneuploids would be greater in the early generations following an intervarietal hybridisation than in a normal variety. Consequently the chromosome numbers were counted of 59 F_2 seedlings from an intervarietal hybrid between Druchamp and Soissonais. Of these 55 had 42 normal chromosomes, one had 42 chromosomes including a telocentric, two were monosomic and one was trisomic. There was thus an aneuploid frequency of 6·8 per cent., which is much higher than the frequency found in any of the varieties studied. The greater pairing failure observed by Person in heterozygous material is probably directly reflected in a greater production of aneuploids.

It is of interest that wheat, an inbreeder, apparently shows increased meiotic irregularity on outbreeding whereas rye, an outbreeder, shows increased irregularity on inbreeding (Rees, 1955). The stability of meiosis presumably depends, therefore, upon balanced genetical systems comparable with those demanded for the stable development of other characters (Jinks and Mather, 1955).

The return to homozygosity following intervarietal hybridisation in wheat will be hastened by the greater failure of pairing and aneuploid production in heterozygous material. In the euploids derived from early generation monosomics the chromosome pair which had passed through the monosomic phase will be homozygous. Moreover the failure of meiotic pairing must diminish the anticipated amount of recombination in wheat hybrids.

These are considerations which may be relevant in the organisation of wheat hybridisation programmes. Thus, because of pairing failure and monosomic production, some chromosomes must be transferred unaltered from one parent into the derived breeding material. This means that some wheat hybridisation programmes involve the unconscious use of procedures somewhat similar to the intervarietal chromosome substitution methods developed by Sears, Loegering and Rodenhiser (1957) and Unrau (1958). However, instead of a chromosome being transferred unaltered from one variety to another, an unmodified chromosome will be transferred from one variety into new genotypes resulting from the recombination and segregation of the rest of the complement. The presumed occurrence of this process draws attention to the complete lack of information, in wheat breeding, on the relative significance of intra-chromosomal recombination and inter-chromosomal segregation.

(iv) *Extraction of monosomic series*

Significant cytogenetic information has been obtained in wheat from manipulations based on aneuploids. The most extensive work has involved the use of the complete series of 21 monosomic lines originally developed in the variety Chinese Spring by Sears (1953). From this original series full sets of monosomics have been produced in other varieties by backcrossing (Unrau, 1958).

The first series of monosomics was extracted from the derivatives of two Chinese Spring haploids and from various Chinese Spring-rye combinations. However, it is clear that given sufficient effort it might well be possible to assemble a series of monosomics in any wheat variety simply by collecting those which occur spontaneously.

This is no longer necessary in *T. aestivum* since it is probably easier to transfer the monosomic condition from one variety to another by backcrossing. Nevertheless the extraction of a monosomic set by selecting spontaneous monosomics might be possible in other species having similar cytogenetic compositions. Such a species is the hexaploid cultivated oat of commerce, *Avena sativa* ($2n = 42$), which has a diploid-like meiotic process in which 21 bivalents are formed. However, its meiosis is not completely regular and, as in wheat, single pairs of chromosomes occasionally fail to form bivalents (Joshi and Howard, 1955).

The consequence of this irregularity should be the formation of aneuploids, as in wheat. To check this the chromosome numbers

were counted of 631 seedlings of the variety Sun II. Of these 623 had 42 chromosomes, seven had 41 chromosomes and one had 34 chromosomes. The chromosome constitutions of the aneuploids were confirmed at meiosis. It thus appears that the assembly of spontaneous monosomics should be possible. The results accruing from the production of a monosomic series in oats might well be as significant in terms of cytogenetic information, and as valuable in relation to practical breeding techniques, as have been those obtained in wheat.

7. SUMMARY

1. Aneuploids are produced in the progenies of euploid individuals of common wheat, *Triticum aestivum*, due to occasional pairing failure at meiosis.

2. Data are available on the behaviour of univalent chromosomes and on the inheritance of aneuploid conditions in wheat. Consequently it is possible to determine the significance of aneuploidy for the structures of varietal populations.

3. The initial aneuploids—monosomics or trisomics—produce progenies which include euploid plants as well as plants with the parental chromosome constitution. After several generations most of the derivatives of a monosomic or trisomic will have reverted to euploid. Thus there is a slow turn-over of the aneuploid fraction of the population.

4. A small proportion of aneuploids becomes fixed in the population through the production of tetrasomics from trisomics and through the production of plants homozygous for misdivision conditions.

5. From the formulae calculated to describe the population structures it can be concluded that pairing failure occurs less often in megaspore mother cells than in pollen mother cells of euploid plants.

6. The overall frequency of aneuploids in the varieties examined was 1·08 per cent. of the population. This is sufficiently high to merit consideration in the establishment of the standards of genetic purity required in seed stocks of wheat.

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THE POPULATION GENETICS OF "SEX-RATIO" IN *DROSOPHILA PSEUDOOBSCURA*

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1. INTRODUCTION

BENNETT (1958), Barker (1958) and Shaw (1959) have all recently discussed the population genetics of the X-linked condition "sex-ratio" in *Drosophila pseudoobscura*. The purpose of the present paper is to put forward a mathematical model considered more appropriate than that used by Bennett and Shaw, and to examine the subject more closely than did Barker.

The condition "sex-ratio" was first studied in detail by Gershenson (1928) in *Drosophila obscura*, and later by Sturtevant and Dobzhansky (1936) and Dobzhansky (1943) in *D. pseudoobscura*. Sturtevant and Dobzhansky showed that "sex-ratio" is associated with an inversion, and is thus not simple genetically. However, in the present paper the words "gene" and "locus" will frequently be applied to it in order to simplify the mathematical arguments, but its more complex nature should be borne in mind. An early record of a similar condition in *Drosophila affinis* occurs in *The genetics of Drosophila* by Morgan, Bridges and Sturtevant (1925).

Briefly, males carrying the abnormal X-chromosome (X_r) produce few or no sons, but nearly twice as many daughters as are produced by normal males. Gershenson remarked that, in the absence of differential viability, the abnormal chromosome would increase in a population until there were no males left. Sturtevant and Dobzhansky also noted this, but added that "the algebraic analysis of populations containing 'sex-ratio' is difficult, and may best be postponed . . .". They found that the abnormal chromosome was present in apparent equilibrium in natural populations.

Wallace (1948) conducted an extensive series of experiments in which he ran population cages and also attempted to estimate the viabilities of the several genotypes directly. It is the results of these experiments to which Bennett, Barker and Shaw have applied their models.

2. POPULATION MODEL

The study of selection on loci which affect the sex-ratio is made difficult by the relevance of arguments concerning the expenditure incurred by parents in rearing offspring, as has been emphasised by Bodmer and Edwards (1960). In order to avoid these difficulties in the present problem it is necessary to postulate that the individual

expenditures on male and female offspring are equal, and that there is no differential viability amongst the offspring between fertilisation and the end of the period of parental expenditure. These restrictions on the model will be discussed later.

Let the genotypes XX, XX_r, X_rX_r, XY and X_rY have effective frequencies at sexual maturity u, v, w, x and y, where $u+v+w+x+y=1$, and let their relative viabilities be 1, a, b, 1 and c, respectively. Further, let the proportion of males born to X_rY fathers be $(1-t)$. Then the

TABLE 1

Derivation of genotype frequencies

Mating	Frequency	Offspring Viability	XX 1	XX_r a	X_rX_r b	XY 1	X_rY c
XY × XX	xu	...	$\frac{1}{2}$	...	...	$\frac{1}{2}$	...
XY × XX_r	xv	...	$\frac{1}{4}$	$\frac{1}{4}$	...	$\frac{1}{4}$	$\frac{1}{4}$
XY × X_rX_r	xw	...	...	$\frac{1}{2}$	...	...	$\frac{1}{2}$
X_rY × XX	yu	...	...	t	...	$(1-t)$	...
X_rY × XX_r	yv	...	...	$\frac{1}{2}t$	$\frac{1}{2}t$	$\frac{1}{2}(1-t)$	$\frac{1}{2}(1-t)$
X_rY × X_rX_r	yw	...	...	...	t	...	$(1-t)$

relative frequencies of the genotypes in the next generation can be found from table 1. These are given by

$$\begin{aligned}
\mathrm{T}u' &= \tfrac{1}{2}x(u+\tfrac{1}{2}v)\\
\mathrm{T}v' &= a(\tfrac{1}{2}x(w+\tfrac{1}{2}v)+ty(u+\tfrac{1}{2}v))\\
\mathrm{T}w' &= bty(w+\tfrac{1}{2}v)\\
\mathrm{T}x' &= (\tfrac{1}{2}x+(1-t)\,y)(u+\tfrac{1}{2}v)\\
\mathrm{T}y' &= c(\tfrac{1}{2}x+(1-t)\,y)(w+\tfrac{1}{2}v),
\end{aligned}$$

where T is such that $u'+v'+w'+x'+y' = 1$. The effective gene ratios at maturity in the next generation are therefore given by

$$r'_f = \frac{a(\frac{1}{2}x(w+\frac{1}{2}v)+ty(u+\frac{1}{2}v))+2bty(w+\frac{1}{2}v)}{x(u+\frac{1}{2}v)+a(\frac{1}{2}x(w+\frac{1}{2}v)+ty(u+\frac{1}{2}v))}$$

for females and $r'_m = c(v+\frac{1}{2}v)/(u+\frac{1}{2}v)$ for males. The original gene ratios are given by $r_f = (w+\frac{1}{2}v)/(u+\frac{1}{2}v)$ and $r_m = y/x$. From these relations

$$r'_f = \frac{a(\frac{1}{2}r_f+tr_m)+2btr_fr_m}{1+a(\frac{1}{2}r_f+tr_m)} \text{ and } r'_m = cr_f \quad \ldots \quad (1) \text{ and } (2)$$

At equilibrium $r'_f = r_f = r_{ef}$ (say) and $r'_m = r_m = r_{em}$, so that $r_{em} = cr_{ef}$, and hence

$$r_{ef} = \frac{ar_{ef}(\frac{1}{2}+ct)+2bctr^2_{ef}}{ar_{ef}(\frac{1}{2}+ct)+1}.$$

Since r_{ef} is not zero, for then the equilibrium would be trivial,

$$r_{ef} = \frac{a(1+2ct)-2}{a(1+2ct)-4bct}.$$

This is the equilibrium effective female gene ratio at maturity. If there is no differential fertility between genotypes of the same sex, then it is the actual ratio at maturity. The male ratio has already been given by $r_{em} = cr_{ef}$.

The equilibrium gene ratios before zygotic selection, s_{ef} and s_{em}, may be found by putting the ratios after selection in equations (1) and (2) and setting $a = b = c = 1$, whence

$$s_{ef} = \frac{\frac{1}{2}r_{ef}+tr_{em}+2tr_{ef}r_{em}}{1+\frac{1}{2}r_{ef}+tr_{em}} \text{ and } s_{em} = r_{ef}.$$

Since the genotype frequencies may be completely expressed in terms of the gene ratios of the previous generation, as is shown below, it is clear that the problem may be exhaustively examined mathematically in terms of these ratios.

$$\begin{aligned} \mathrm{T}^*u' &= \tfrac{1}{2} \\ \mathrm{T}^*v' &= a(\tfrac{1}{2}r_f+tr_m) \\ \mathrm{T}^*w' &= btr_{fr_m} \\ \mathrm{T}^*x' &= (\tfrac{1}{2}+(1-t)r_m) \\ \mathrm{T}^*y' &= c(\tfrac{1}{2}+(1-t)r_m)r_f. \end{aligned}$$

In particular, the conditions that the equilibrium is stable may be evaluated by examining the properties of the gene ratios when they are near to their equilibrium values. It transpires that the equation for the latent roots of the generation matrix of the gene ratios near equilibrium is

$$\lambda^2-2\lambda\frac{4bct(act-1)+a}{a^2(1+2ct)^2-8bct}-4ct\frac{b(a-2)+a}{a^2(1+2ct)^2-8bct} = 0,$$

the condition for stability being that the absolute values of both roots must be less than 1.

If the factor $2ct$ is regarded as an overall viability, this equation is formally identical to that considered by Bennett, who stated, without proof, that the equilibrium is stable if and only if both the numerator and denominator of the expression for r_{ef} are positive, that is if

$$a(1+2ct)>2 \text{ and } a(1+2ct)>4bct.$$

A proof of this statement follows.

Putting $a(1+2ct) = \mathrm{H}$, $2 = \mathrm{B}$ and $4bct = \mathrm{A}$, $r_{ef} = (\mathrm{H}-\mathrm{B})/(\mathrm{H}-\mathrm{A})$ the notation being specifically chosen to draw an analogy with the autosomal case.

Writing the equation for the latent roots as $\lambda^2+\mathrm{L}\lambda+\mathrm{M} = 0$ it is easy to show that

$$\mathrm{L}+\mathrm{M} = -\frac{\mathrm{B}(\mathrm{H}-\mathrm{A})+\mathrm{A}(\mathrm{H}-\mathrm{B})}{\mathrm{H}^2-\mathrm{AB}},$$

and hence that

$$1+\mathrm{L}+\mathrm{M} = \frac{(\mathrm{H}-\mathrm{A})(\mathrm{H}-\mathrm{B})}{\mathrm{H}^2-\mathrm{AB}},$$

From these relations, if $H>A$ and $H>B$ then $L+M<0$ and $1+L+M>0$, and if $H<A$ and $H<B$ then $L+M<0$ and $1+L+M<0$. Furthermore, the reverse holds true, since $H-A$ and $H-B$ must be of the same sign for the female gene ratio to be positive.

The next stage in the proof is to show that $-1<L<0$ and $-1<M<0$ for all possible values of the viabilities that do not lead to trivial equilibria.

Suppose $H>A$, $H>B$. Then the denominators of L and M, H^2-AB, are positive. Further, since $a(1+2ct)>4bct$ and $a(1+2ct)>2$, $a/b>4ct/(1+2ct)$ and $a>2/(1+2ct)$. Hence $a+a/b>2$, or $(a-2)+a/b>0$. Therefore the numerator of L, $-4ct(b(a-2)+a)$, is negative, and $L<0$.

Similarly, since $a(1+2ct)>2$, $4ct(act-1)>-4ct/(1+2ct)$. Hence $4ct(act-1)+a/b>0$; the numerator of M, $-2(4bct(act-1)+a)$, is negative, and $M<0$.

Again, clearly

$$a(1+2ct)+2act(1+2ct)>4bct+4ct,$$

or

$$a(1+2ct)^2-4bct-4ct>0,$$

and hence $a^2(1+2ct)^2-8bct-4ct(b(a-2)+a)>0$. Thus the numerator of the fraction $1+L$ is positive, so that $1+L>0$ and $L>-1$.

Similarly, since

$$a(1+2ct)+2act(1+2ct)>2+8bc^2t^2,$$

$$a(1+2ct)^2-2-8bc^2t^2>0$$

and

$$a^2(1+2ct)^2-8bct-2(4bct(act-1)+a)>0.$$

Thus $1+M>0$ and $M>-1$.

When $H<A$, $H<B$, the same relations hold but with the inequality signs reversed, so that the numerators of L and M are positive. But since then $H^2<AB$, the denominators are negative, and $L<0$ and $M<0$ are still true. Similarly, $L>-1$ and $M>-1$, so that for all values of the viabilities leading to non-trivial equilibria

$$-1<L<0 \text{ and } -1<M<0.$$

Now the condition for stability is $\left|-L\pm\sqrt{L^2-4M}\right|<2$. Knowing the possible values of L and M, it is apparent that an equivalent condition is

$$-L+\sqrt{L^2-4M}<2,$$

whence

$$\sqrt{L^2-4M}<2+L,$$

$$L^2-4M<4+4L+L^2,$$

and

$$1+L+M>0.$$

Hence $H>A$, $H>B$ are necessary conditions for stability. The

condition for instability is similarly

$$-L+\sqrt{L^2-4M}>2,$$

whence $$1+L+M<0.$$

Therefore $H<A$, $H<B$ are necessary conditions for instability, and hence $H>A$, $H>B$ are necessary and sufficient conditions for stability.

3. APPLICATION TO WALLACE'S DATA

This model may now be applied to Wallace's data.

Wallace's experiments were of two kinds. First, he ran four population cages, two at 16½° C. and two at 25° C., in which the foundation stocks consisted of known numbers of the several genotypes. Samples of eggs were taken from these cages every month or so, and assayed in order to determine the frequencies of the genotypes. Secondly, in a series of subsidiary experiments, he made quantitative estimates of the following components of fitness of the different genotypes: larval competition; longevity; fecundity (female); sexual activity (male); and egg hatchability (female). By multiplying these estimates together he obtained an overall selective value for each genotype, and he quoted the maximum and minimum values that he considered possible at each temperature. These viabilities were given relative to that of the normal in males and of the heterozygote in females. Wallace multiplied his values for the relative viabilities of the abnormal males by a factor of two in an attempt to express the selective advantage due to the abnormal sex ratio amongst their offspring. However, this advantage has already been allowed for in the present model, so that it is necessary to remove this factor of two before using these values. Wallace's mean viabilities at each temperature, expressed relative to that of the normal in each sex, and with the factors of two omitted, are given in table 2. Darlington and Dobzhansky (1942) studied the effect of temperature on the parameter t and found a value of 0·9382 at 25° C. and a value of 0·9878 at 16½° C. The equilibria, which are stable, corresponding to these values of a, b, c and t are given in table 3.

In Wallace's population cages the X_r chromosome was lost completely at 25° C. (and hence the gene ratios were all zero), whilst at 16½° C. the following gene ratios were obtained from samples of eggs taken after approximately twelve generations of selection (when the experiment was ended):

Cage 12. Female: 0·062; Male: 0·095.
Cage 13. Female: 0·048; Male: 0·063.

Even when the sizes of the egg samples are taken into account it is apparent that these observed ratios are incompatible with the theoretical equilibrium ratios. It must therefore be concluded that either the present model is inadequate, or Wallace's values for the overall

viabilities differ from the viabilities which actually pertained in the population cages. Partial vindication of the model could be obtained by seeing if viabilities exist that would make the model populations approach the observed equilibria at the correct rates. But before doing this it is appropriate to examine the limitations of the model, of which there are four main ones:

(1) Population and progeny sizes are assumed infinite.
(2) Generations do not overlap.
(3) Parental expenditures on individuals of either sex are set equal.
(4) Viability is assumed not to be sex-differential during the period of parental expenditure.

TABLE 2

Wallace's mean viabilities

Temperature	Genotypes				
	XX 1	XX_r *a*	X_rX_r *b*	XY 1	X_rY *c*
25° C.	1	3·384	0·059	1	0·355
16½° C.	1	1·263	0·392	1	0·711

TABLE 3

Equilibrium gene ratios with Wallace's viabilities

Temperature	Before genotypic selection		After genotypic selection	
	Female	Male	Female	Male
25° C.	0·537	0·654	0·654	0·232
16½° C.	0·637	0·536	0·536	0·381

That the first limitation is unimportant is shown by Barker's computations on an electronic digital computer, by which he compared the changes in gene frequency due to selection in infinite populations and populations the size of Wallace's. Although, as will be mentioned, Barker's work is open to criticism in some respects, the comparisons remove any doubt that the assumption of infinite population and progeny sizes is important in this instance.

The fact that the model does not include the overlapping of generations is unlikely to matter very much, especially in the present context where there are so few generations.

The third limitation is also reasonable: it is unlikely that parental

expenditure is significantly sex-differential in *Drosophila* species, since sexual dimorphism is not noticeable until well after parental expenditure must have ceased, and also since the normal sex-ratio is about one-half.

As far as the fourth limitation is concerned, in *Drosophila* all the fertilised eggs are laid whether they are viable or not, so that sex-differential viability before the end of the period of parental expenditure does not exist. It could be argued that parental expenditure on inviable eggs differs from that on viable eggs, but, as Gershenson showed, such a small proportion of eggs is inviable that this could hardly be important.

It is thus apparent that, given correct viabilities, the model should be a good approximation to reality, and it must be concluded that Wallace's viabilities are inadequate estimates of the relative selection pressures involved.

In order to discover whether reasonable values for the viabilities exist which would make the model compatible with Wallace's experimental findings, the original recurrence relations in the genotype frequencies were programmed onto EDSAC II, the Cambridge University Mathematical Laboratory's high-speed electronic digital computer. The following variables could be set at the start of the programme:

u, v, w, x and y, the initial genotype frequencies;
a, b and c, the relative viabilities;

and t, the proportion of females born to X,Y males.

The output, which could be printed to any reasonable number of figures, consisted of the following information, printed every nth generation, where n could be set beforehand:

the relative frequencies of the five genotypes;
the sex ratio;
the gene frequencies in males, females, and in the whole population;
and "T".

The programme could be set to print this information either before or after genotypic selection, and to run for any number of generations.

It was not considered worthwhile to write a comprehensive programme which would search for the viabilities which give the best fit to Wallace's selection data. Thus there may be better values than those finally arrived at, so that the viabilities quoted below cannot be considered proper estimates. Nevertheless, the investigation showed that it is possible to simulate Wallace's results satisfactorily, with certain reservations.

Figs. 1, 2, 3 and 4 show the simulated selection response curves together with the 95 per cent. fiducial limit curves, in terms of genotype frequencies before zygotic selection, for Cages 10, 11, 12 and 13.

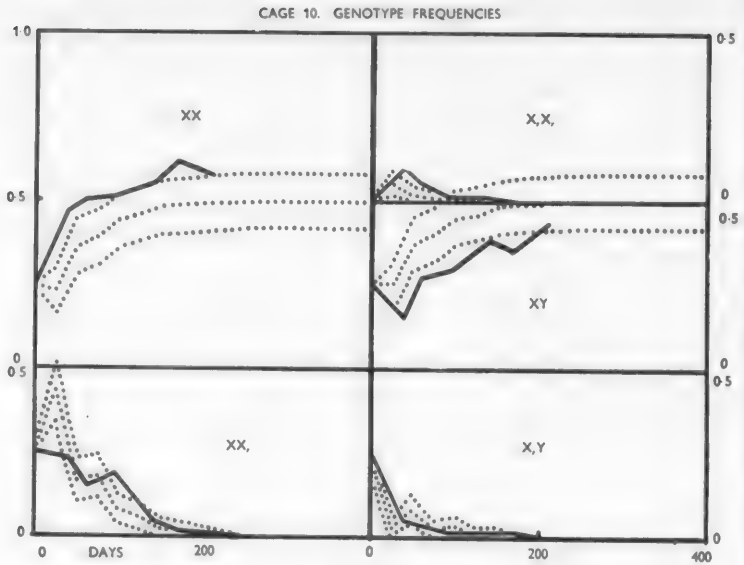
CAGE 10. GENOTYPE FREQUENCIES
1·0
0·5
0
XX
X,X,
XY
XX,
X,Y
0·5
0
DAYS
200
400

FIG. 1.

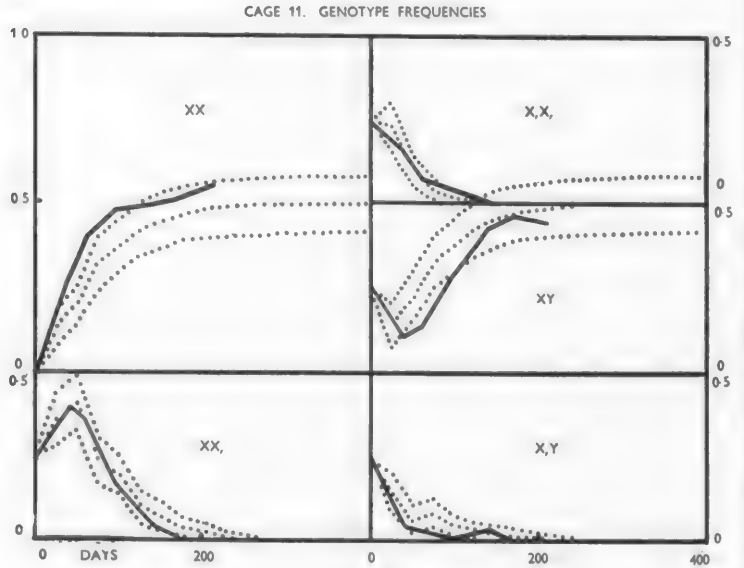
CAGE 11. GENOTYPE FREQUENCIES
1·0
0·5
0
XX
X,X,
XY
XX,
X,Y
DAYS
200
400

FIG. 2.

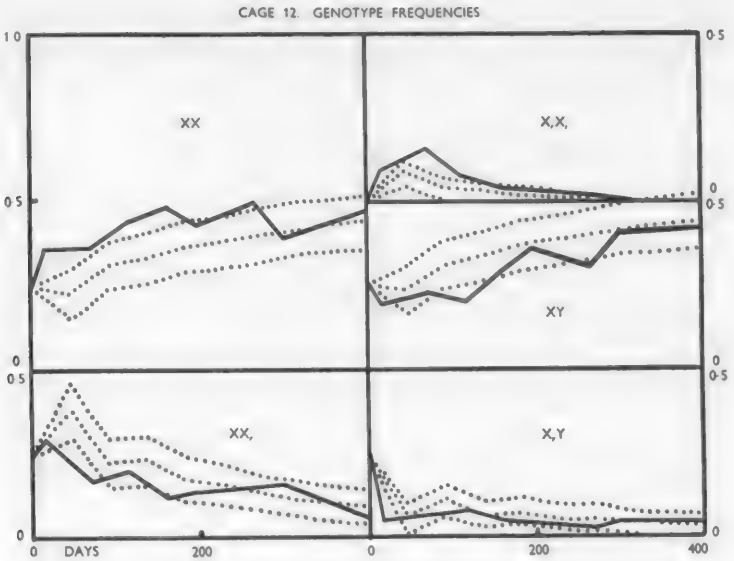
CAGE 12. GENOTYPE FREQUENCIES
1·0
0·5
0
XX
X,X,
XY
XX,
X,Y
DAYS
200
400

FIG. 3.

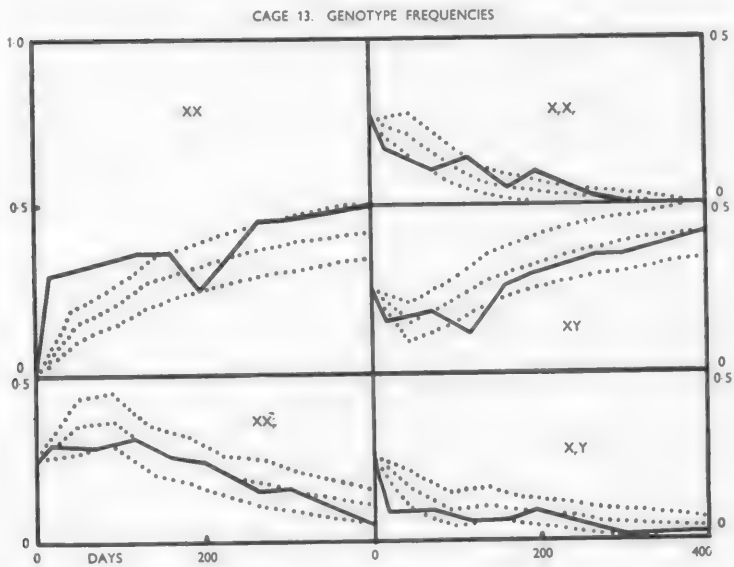
CAGE 13. GENOTYPE FREQUENCIES
1·0
0·5
0
XX
X,X,
XY
XX,
X,Y
DAYS
200
400

FIG. 4.

Cages 10 and 11 had been maintained at 25° C., so that t was taken to be 0·9382 for them, and Cages 12 and 13 at 16½° C., for which t was taken to be 0·9878. The simulated curves were obtained using the following viabilities:

Cages 10 and 11: $a = 0{\cdot}3$; $b = 0$; $c = 0{\cdot}95$;
Cages 12 and 13: $a = 0{\cdot}7$; $b = 0$; $c = 0{\cdot}7$.

The viabilities at 25° C. correspond closely to those suggested by Barker (0·4, 0 and 0·95), which were indeed used as a first approximation.

In each of the four cages the simulated response curves agree well with those obtained experimentally by Wallace, except that the predicted frequency of XX individuals is consistently too high, whereas that of XY individuals is too low. On the present model it is easy to show that, whatever the viabilities, the expected frequency of XX must be less than that of XY except when the X_r chromosome has been eliminated, in which case the frequencies are equal. But in practice the reverse is true: it seems as though the assumption that normal individuals breed with a sex-ratio of one-half is false, and this is borne out by Wallace's populations kept at 25° C., in which the following numbers of male and female progeny were counted after X_r had apparently been eliminated:

Cage 10: 64 males and 86 females;
Cage 11: 67 males and 83 females.

There is clearly no heterogeneity between the cages, and the combined sex-ratio of 131 : 169 is significantly different from 1 : 1 at the 5 per cent. level. Evidently a model which took this abnormal sex ratio into account would fit the data even more closely than the present one. Wallace did not comment on this sex-ratio, but Gershenson, who is the only other writer on the subject to quote comparable sex-ratios, found no deviation from 1 : 1. It therefore seems likely that this abnormality is confined to Wallace's experimental populations, and is perhaps due to a factor such as overcrowding.

As can be seen from the figures, the simulated selection curves approach fixation of the normal gene at both temperatures, so that the apparent equilibria of Cages 12 and 13 are probably fortuitous.

4. ESTIMATION OF VIABILITIES IN NATURAL POPULATIONS

It is interesting to investigate what viabilities would give rise to an equilibrium X_r chromosome frequency of p.

Working in terms of gene ratios at maturity,

$$r_{ef} = \frac{a(1+2ct)-2}{a(1+2ct)-4bct}$$

and

$$r_{em} = cr_{ef}.$$

Dobzhansky reported that there was no significant difference between the male and female gene ratios in any sample from the natural populations he studied, so that $r_{em} = r_{ef}$ and $c = 1$. t may be taken to be equal to 1, whence

$$r_{em} = r_{ef} = p/(1-p) = (3a-2)/(3a-4b).$$

Supposing that the inviability effect of the X_r chromosome is additive, $(1-b) = 2(1-a)$, or $2a-b = 1$.

Hence $$a = (2p+2)/(2p+3)$$

and $$b = (2p+1)/(2p+3).$$

Table 4 gives some examples of values of p, a and b that satisfy these relations. These viabilities lead to stable equilibria. Sturtevant and Dobzhansky, and Dobzhansky, reported gene frequencies ranging from

TABLE 4

Equilibrium gene frequencies corresponding to various viabilities

Viabilities			Gene frequency
a	b	c	p
0·67	0·33	1	0·0
0·69	0·38	1	0·1
0·71	0·41	1	0·2
0·72	0·44	1	0·3
0·74	0·47	1	0·4

zero to 30 per cent. in natural populations of *Drosophila pseudoobscura*, and the suggested viabilities could account for these. Moreover, small changes in the viabilities lead to relatively large changes in the gene frequencies, so that both the wide range of apparent equilibria and the changes in gene frequency with time are to be expected.

5. CRITICISM OF PREVIOUS WORK

It was mentioned in the introduction that previous treatments of this subject have not been entirely satisfactory. The first attempt at a model for the "sex-ratio" locus was made by Bennett. He put forward a model appropriate to a sex-linked locus without gametic selection, but then committed the error of applying this model to the present case, in which gametic selection, or an equivalent phenomenon, occurs. In effect he confounded gametic and zygotic selection, an erroneous procedure in this case, as will be shown below.

Unfortunately it happens that his results are, on the surface, reasonable, and some are indeed correct. For example, the characteristic equation for the approach of the gene ratios at maturity to their equilibrium values contains c and t, the two viabilities in question,

always confounded as the product ct, so that the rate of approach near equilibrium, and the stability of the equilibrium, are correctly given by Bennett's equations. Similarly, the equilibrium gene ratio at maturity in females is correct, but that in males is wrong because in the expression for it c occurs unaccompanied by t. As an example of the erroneous results from Bennett's model, the equilibrium situation at maturity using Wallace's maximum selective values at 25° C. is

TABLE 5

Equilibrium frequencies at maturity on the two models

	Bennett	Edwards
XX	0·149	0·172
XX_r	0·528	0·612
X_rX_r	0·003	0·003
XY	0·218	0·172
X_rY	0·102	0·040
Sex-ratio	0·320	0·212
Male gene frequency	0·319	0·190
Female gene frequency	0·393	0·393
Overall gene frequency	0·379	0·369

given for the two models in table 5, it being presumed in both cases that X_rY males produce no sons at all.

In the case of results given before zygotic selection, Bennett's model leads to the correct gene ratios in males and females at each generation, and hence at equilibrium, but since the sex-ratio of the population is, on his model, then always one-half, the overall gene ratio is incorrect. The genotype frequencies are also wrong.

TABLE 6

Equilibrium frequencies at fertilisation on the two models

	Bennett	Edwards
XX	0·207	0·246
XX_r	0·231	0·274
X_rX_r	0·063	0·075
XY	0·304	0·246
X_rY	0·196	0·159
Sex-ratio	0·500	0·405
Male gene frequency	0·393	0·393
Female gene frequency	0·356	0·356
Overall gene frequency	0·368	0·365

Table 6 gives a comparison similar to that in table 5, but with frequencies before genotypic selection. In view of the inapplicability of Bennett's model to the present problem it seems best to disregard it in this connection.

Barker investigated the problem stochastically on an electronic computer, and in his programme he made allowance for both gametic and zygotic selection individually. He also ran a programme, for

comparison, in which the two forms of selection were confounded, as in Bennett's model, and concluded that in his work " the methods of simulation of selection used are adequate and that specific or overall [confounded] selective values may be used to study any given genetic situation ". He was misled into making the latter part of this statement by the fact that he happened to be working only in terms of the gene frequencies in males and females before zygotic selection, which, as has been stated above, are the same in both models. Had he investigated the genotype frequencies, or the sex ratio, discrepancies would have been apparent. So far as his own model goes, conclusions drawn from it seem to be valid.

Shaw contributed a short article on the problem, in which he did not appreciate Bennett's errors. He agreed with Bennett that the equilibrium male gene frequency at maturity, using Wallace's maximum viabilities at 25° C., was " about 30 per cent.", whereas the correct value is 19 per cent. (see table 5). He also stated that " once the equilibrium frequency of the " sex-ratio " males is known, that of the other genotypes is fully determined ". This statement is only true if one also knows the sex ratio.

Sprott (1957) set up a model for any number of alleles at a sex-linked locus, and, working in terms of the male and female gene frequencies, established the conditions for stable equilibrium. He did not apply this model to any real situation. His stability conditions are no more than a slight simplification of the determinantal equation for the latent roots of the generation matrix, and are not given as simple inequalities in the viabilities. He gave an example of a stable equilibrium for two alleles in which the gene frequencies agree with those calculated by the method given in the present paper. However, he gave the corresponding female genotype frequencies incorrectly, owing to the fact that he did not appreciate that he was working in terms of the gene frequencies *after* selection, as is clear from his original equations. The female genotype frequencies he quoted are those before selection.

6. SUMMARY

In *Drosophila pseudoobscura* the X-linked condition " sex-ratio " causes males carrying it to produce few or no sons, but nearly twice as many daughters as are produced by normal males (Gershenson, 1928; Sturtevant and Dobzhansky, 1936). In the absence of differential viability one might expect it to increase in a population until there were no males left, yet equilibria, with gene frequencies up to 30 per cent., have been found in natural populations (Sturtevant and Dobzhansky, 1936; Dobzhansky, 1943). Wallace (1948) conducted a series of experiments to determine the viabilities, and set up some population cages to follow the progress of the gene. In these cages the gene was either eliminated, or was at a very low frequency when the experiment was ended. Bennett (1958) put forward a mathematical

model for a sex-linked locus which he applied to Wallace's findings, but examination of his model reveals an error which makes it inapplicable to this situation: he confounded gametic and genotypic viabilities, a course which invalidates some of his findings. The present paper describes a more appropriate model, which has been used to study the polymorphism. It is shown that Wallace's viabilities fit neither the natural nor his experimental populations, and new viabilities are suggested for each case.

Acknowledgments.—I wish to thank the Director of the Mathematical Laboratory, University of Cambridge, for permission to use EDSAC II, the high-speed electronic digital computer, and Drs W. F. Bodmer and K. E. Machin for helpful discussions on the proof of Bennett's stability conditions.

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THE TRANSPLANTATION OF NUCLEI BETWEEN TWO SUBSPECIES OF *XENOPUS LAEVIS*

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1. INTRODUCTION

THE technique of nuclear transplantation enables an embryonic nucleus from one kind of frog to be combined with the egg-cytoplasm from another kind. The extent to which the resulting transplant-embryos can differentiate varies according to how closely related are the species or subspecies from which the nucleus and cytoplasm are derived. Embryos, tadpoles, and frogs derived from any combination of nucleus and cytoplasm from different species or subspecies are described as " heterotypic ". In the experiments described below, nuclei have been transplanted between two subspecies of *Xenopus laevis*, which are sufficiently closely related for some of the resulting transplant-embryos to grow into adult frogs. These show the result of interaction between the nucleus and cytoplasm during the differentiation of the characters which distinguish the subspecies. The transfer of nuclei from advanced donor stages into egg-cytoplasm of the foreign subspecies indicates how similar is the rate, and time of onset, of nuclear differentiation in the two subspecies; these results also indicate the significance of the considerable difference in these respects between *Xenopus laevis* and *Rana pipiens* (Gurdon, 1960*c*). Lastly, frogs derived from the serial transfer of nuclei between the subspecies show whether nuclei undergo autonomous differentiation or suffer any damage as a result of continued transplantation.

2. MATERIAL AND METHODS

The frogs used in these experiments belong to the two subspecies *Xenopus laevis laevis* (Daudin) and *Xenopus laevis victorianus* Ahl. The stock individuals of *X. l. victorianus* were reared in the laboratory from tadpoles kindly made available by Professor Newth, and obtained from Entebbe, Uganda. The *X. l. laevis* frogs used were reared in the laboratory from specimens originally obtained in S. Africa. The main differences between the subspecies concern size and colour pattern, and are described below (p. 308). Hybrids obtained by reciprocal crosses between the two subspecies are viable and fertile, and more or less intermediate in character (Blackler, Fischberg and Newth, in preparation).

Throughout these transplantation experiments, donor nuclei from the vegetal hemisphere or endoderm region have been used since these are easier to handle than nuclei from other embryonic regions. Eggs were obtained by injecting females with gonadotropic hormone.

The transplantation technique employed has been described elsewhere (Elsdale,

Gurdon and Fischberg, 1960 ; Gurdon, 1960*b*). The technique involves the injection of an embryonic cell, the wall of which has been broken, into an unfertilised and enucleated egg. It mainly differs from that of Briggs and King (1953) in two respects. First the recipient eggs of *Xenopus* are irradiated with ultraviolet for about one minute. This treatment kills the egg-nucleus which then degenerates in the egg-cytoplasm, but the latter is not damaged in any way (Gurdon, 1960*a*). Briggs and King, on the other hand, remove the egg-nucleus with a needle from the recipient eggs of *Rana pipiens*. Secondly, the *Xenopus* nuclear marker (Elsdale, Fischberg and Smith, 1958) was used, which provides proof that only the injected nucleus has participated in the development of the transplant-embryo. The marker behaves as a mutation which suppresses the formation of the nucleolus from one haploid set of chromosomes ; the application of its use for transplantation experiments has been described elsewhere (Elsdale, Gurdon and Fischberg, 1960).

3. RESULTS

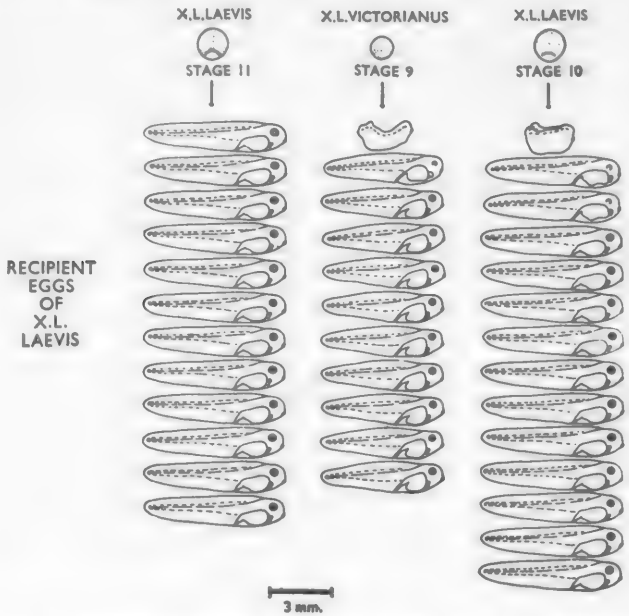
(i) *The early development of heterotypic transplant-embryos*

Text-figs. 1 and 2 consist of camera lucida drawings of donor embryos and the transplant-embryos derived from them. In these experiments nuclei from both subspecies have been transplanted into recipient eggs of *laevis*; all transplantations in text-fig. 1 were made into the eggs of one frog, and all those in text-fig. 2 into the eggs of another. Twenty-three nuclei were transplanted from each donor embryo, but only those transplant-embryos are shown which became regular late blastulae. Those transplantations which failed to cleave at all or which cleaved irregularly are not shown, since this abnormality may be due, in some cases at least, to the technical treatment connected with transplantation (Gurdon, 1960*b*). The number of embryos underneath each donor therefore represents the proportion of transplanted nuclei out of 23 which gave rise to regular late blastulae. Transplant-embryos were not kept beyond the early feeding tadpole stage, since it has been found from previous experience that at least 95 per cent. of embryos which reach this stage can be easily reared into adult fertile frogs. In these diagrams, therefore, at least 95 per cent. of all *feeding* tadpoles represent the presence in the original donor embryo of undifferentiated and totipotent nuclei.

Text-fig. 1 shows the results of transplanting blastula and gastrula nuclei. The embryos derived from *laevis* mid-gastrula nuclei are all normal, and those in the other two series are nearly all normal. This is the kind of result that has previously been obtained from undifferentiated nuclei of *laevis* blastulae and gastrulae (Gurdon, 1960*c*). The conclusion from these experiments is that there is no difference in the developmental capacity of early donor stage nuclei of *laevis* and *victorianus*, and hence that blastula and gastrula nuclei of both subspecies are still undifferentiated.

Text-fig. 2 illustrates experiments involving the transplantation of nuclei from more advanced donor stages of *laevis* and *victorianus*. The quality of eggs laid by frogs kept in the laboratory is very variable, and it is necessary to find out whether abnormal transplant-embryos represent differentiated nuclei or merely recipient eggs incapable of

normal development. In text-fig. 2, the embryos derived from stage 28 nuclei of *laevis* are nearly all normal. As the eggs laid by a frog at any one ovulation are usually of consistent quality, this indicates that the abnormalities obtained after transfer of nuclei from the stage 37 and older donor stages are in this case due to nuclear differentiation and not to the poor quality of the recipient eggs. Nuclei from hatched tadpoles of both subspecies (1st, 2nd and 5th series of text-fig. 2) gave very similar results.

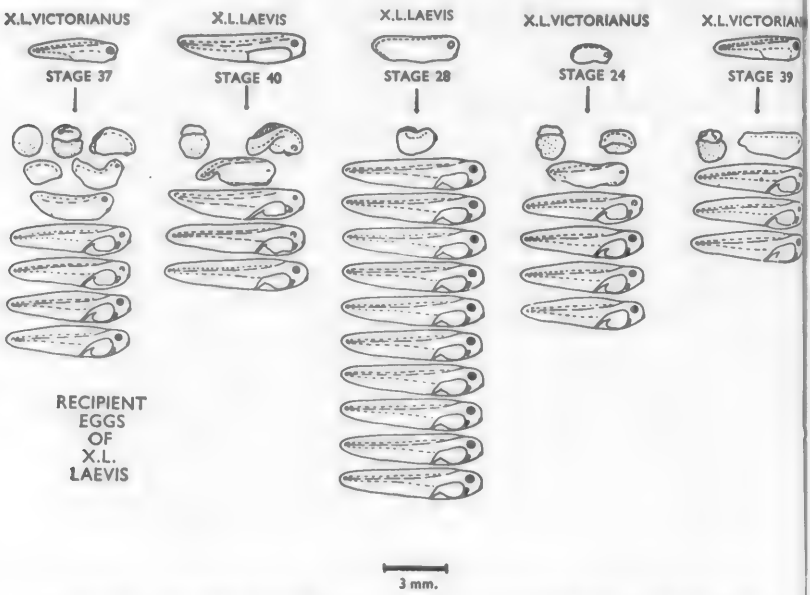


TEXT-FIG. 1.—Camera lucida drawings of embryos derived from the transplantation of nuclei from a mid-gastrula (*laevis*), a late blastula (*victorianus*), and an early gastrula (*laevis*), all into recipient eggs laid by one *laevis* female. Twenty-three transplantations were made in each of the three series, but only those transplant-embryos which cleaved regularly are shown.

Two conclusions can be drawn from the experiments illustrated in text-figs. 1 and 2. First, the rate of differentiation of endoderm nuclei is very similar in both subspecies. Second, there is no incompatibility preventing the nuclei of one subspecies from cleaving regularly and forming normal tadpoles when combined with the cytoplasm of the other subspecies.

Reciprocal experiments have also been carried out in which nuclei from early and advanced stages of both subspecies were transplanted into recipient eggs of *victorianus*. It was found that the nuclei of both subspecies gave very similar results, but that the development of transplant-embryos from all donor stages was much more abnormal

with the recipient eggs of *victorianus* than with those of *laevis*. This result could be due to the variable quality of *victorianus* eggs, and perhaps also to their smaller size (1 mm. diameter, compared to 1·5 mm. for *laevis* eggs). On the other hand it might indicate that the development of transplant-embryos varies according to the type of recipient egg used. It is not yet possible to distinguish between these possibilities, since the smaller number of eggs laid, over a shorter period of time, by *victorianus* females made it impossible to obtain adequate control series, with which to assess egg-quality.



TEXT-FIG. 2.—See legend to text-fig. 1, which is directly applicable, except that donor nuclei were taken from the endoderm of a hatched tadpole of *victorianus*, a swimming tadpole and a tail-bud stage of *laevis*, and a post-neurula and swimming tadpole of *victorianus*. Again 23 transplantations in each series.

(ii) *The differentiation of subspecific characters in heterotypic transplant-animals*

Plate IA shows certain stages in the development of *victorianus* controls (left-hand column), *laevis* controls (right-hand column), and the two heterotypic combinations (centre columns). The top row (A) consists of blastulae which indicate the original egg size. *Victorianus* eggs are less than $\frac{1}{3}$ of the volume of, and much less pigmented than, *laevis* eggs, and this difference in size persists up to the time when the tadpoles begin feeding (row F). This shows that a nucleus can participate in normal development with an initially much greater or much smaller volume of cytoplasm than is usual for it.

Apart from this difference in size, the first clear distinction between the subspecies concerns the appearance of body melanophores; in *laevis* these appear before hatching (row C), while in *victorianus* they do not appear until the swimming tadpole stage (row E). In the heterotypic combinations the appearance of these melanophores is entirely nucleus-controlled. They can be seen before hatching in *laevis* nuclei with *victorianus* eggs, but do not appear until the swimming tadpole stage in *victorianus* nuclei with *laevis* eggs (row E). Another clear difference between the subspecies is the stage at which anal melanophores appear. These melanophores are first seen in *victorianus* very soon after feeding commences (Nieuwkoop and Faber's (1956) stage 48-49), while in *laevis* they do not appear until the tadpoles have already been feeding for 1 to 2 weeks (stage 57). In heterotypic tadpoles this character again develops just as in the subspecies which contributed the nucleus. Other differences between the subspecies are described elsewhere (Blackler, Fischberg and Newth, in preparation). In tadpoles, these concern the posterior edge of the endoderm at stage 40, the angle between the anal tube and gut at stage 41, and the shape and depth of the tail fin in advanced feeding tadpoles. At metamorphosis there is a clear difference in the shape of the resorbing tail, and later in the shape of the forearms. After metamorphosis the colour and pattern of the two types of frogs is quite distinct. *Victorianus* frogs are a light yellowish-green, and gradually acquire a very fine freckling of dark green or black as they reach maturity. *Laevis* frogs, on the other hand, have a darker green or blackish ground colour, with pronounced black mottling. As *victorianus* reach maturity, the underside of the hind-legs develops a yellow coloration which is bordered on the outer side by an irregular band of blackish spots. This pattern is not present in *laevis*. The latter are much bigger frogs than *victorianus*; when adult, the average weight of laboratory reared *laevis* females and males is 75 and 45 grams respectively, while that of *victorianus* females and males is 15 and 8½ gms. In all these respects the heterotypic embryos, tadpoles, and frogs are indistinguishable from controls of the subspecies from which their nuclei were derived.

Plate II contains photographs of adult frogs, all of which are males. Frogs derived from *victorianus* nuclei and *laevis* cytoplasm (top left) are identical to control *victorianus* (lower left), and show none of the characters of the *laevis* control (middle left). The right-hand side of the plate shows a serial transplantation experiment. Original donor nuclei from a *victorianus* late gastrula were transplanted into *laevis* recipient eggs. When the resulting embryos became late gastrulae, one of them was used to provide nuclei for further transplantations into other *laevis* eggs. The remaining gastrula transplant-embryos were allowed to develop further, some of them becoming adult frogs (as shown). This procedure was repeated three times so that the nuclei that have given rise to the three frogs shown at the bottom

right-hand side of the picture have had to synthesise and replicate in foreign cytoplasm for four " gastrula generations "—that is, for over 60 divisions or for 45 divisions in excess of those that take place in the normal development of an individual. In spite of this, the nuclei have been entirely unaffected by the foreign cytoplasm, since the 4th transfer frogs are indistinguishable from *victorianus* controls. These 4th transfer frogs (bottom right) were mated to control *victorianus* females; they proved to be as fertile as controls reared in the laboratory and their offspring were entirely of the *victorianus* type. This constitutes further evidence that the nuclei were unaffected by the foreign cytoplasm, since the male gametes of these frogs carried none of the *laevis* characters; the latter should be easily observable since hybrids between *laevis* and *victorianus* are intermediate in character, as mentioned above.

It should be mentioned here that the adult frogs shown in plate II have been selected as the largest individuals obtained. In the transplantation series concerned, other frogs were also obtained, but these only differed from those shown in that they were smaller. The retardation of their growth seems clearly to be a consequence of the laboratory rearing conditions (Gurdon, 1961). The selection of frogs for plate II is therefore only a question of having chosen those individuals which matured first, and does not in any way affect the conclusions.

A further series of experiments have been carried out in which nuclei from original *laevis* donors were transferred to *victorianus* cytoplasm; some of these were then serially transferred back to *laevis* eggs for 2 gastrula generations, while others were transferred again into *victorianus* eggs. At each stage in the experiment, some transplant-embryos were kept for rearing up into adult frogs, the most fully grown of which are shown in plate Ib. Irrespective of the number of divisions that their nuclei have made in foreign cytoplasm, or in their own kind after back-transfer, the resulting frogs were typical *laevis* in every respect. This confirms the results above, that there is, with very rare exceptions, complete nuclear specificity in the expression of all distinguishing characters so far studied in these two subspecies.

4. DISCUSSION AND CONCLUSIONS

(i) *The differentiation of distinguishing characters in individuals resulting from heterotypic combinations of nucleus and cytoplasm*

No attempt will be made to review the literature concerning this question but one or two examples will be quoted to indicate the main kinds of results that have been obtained. This problem has been extensively investigated by fertilising enucleated eggs with sperm from a different species or subspecies; the resulting embryos, which are haploid, die at an early embryonic stage characteristic for the particular combination concerned (Baltzer, 1952). It is only with relatively closely related forms that such hybrid merogons are at all viable, but the distinguishing characters do not usually become evident

before the tadpoles die owing to their haploid condition. This difficulty has been partially overcome by transplanting certain tissues from the haploid hybrid merogon to a normal diploid host, where they can differentiate further, showing up specific characters (*e.g.* Hadorn, 1937; Dalton, 1946). Most of the characters which have been studied by these means differentiate just like those of the species or subspecies which provided the nucleus. On the other hand cytoplasmic control of the expression of certain characters has also been recorded (Hadorn, 1937).

In more recent years, this general problem has been investigated using the technique of nuclear transplantation which avoids the inviability associated with the haploid condition. In the case of nuclear transfers between different species or genera, the resulting transplant-embryos have always been unable to survive up to a stage when distinguishing characters become apparent. However, Sambuichi (1957) transplanted nuclei between two subspecies of *Rana nigromaculata* and found that in all characters observed the heterotypic transplant-embryos developed like the "nuclear subspecies"; McKinnell (1960) also observed an entirely nucleus-specific expression of a single character by which two races of *Rana pipiens* differ.

The experiments with *Xenopus* subspecies described above are consistent with previously published results, in that all the characters studied which affect embryonic and larval development as well as adult frogs were controlled entirely by the nucleus and not by the cytoplasm. Only direct consequences of the volume of egg-cytoplasm resulted in a cytoplasmic effect on development. It is not certain whether the egg-cytoplasm of the two subspecies is qualitatively different or only quantitatively so. However, the fact that the nucleus of one subspecies is able to express itself perfectly in foreign cytoplasm shows that there is no nucleo-cytoplasmic incompatibility between the subspecies, and that the nucleus is not affected in its function by such differences as do exist in the two kinds of egg-cytoplasm.

Another matter, to which these results are relevant, concerns what effect, if any, foreign cytoplasm has on nuclei synthesising from it. Astaurov and Ostriakova-Varshaver (1957) have made diploid hybrid merogons between two types of silk-moth and have then back-crossed them to the same type as that which provided the nucleus of the merogon. In this way they exposed the nuclei of one type to the cytoplasm of the other for one whole generation, but found that they had been entirely unaffected by this.

By means of serial nuclear transplantation in Anura it is possible to transfer a nucleus to foreign cytoplasm and then, when it has synthesised there for several mitoses, to return it to its own kind of cytoplasm. A comparison of the resulting back-transfer embryos with controls whose nuclei have been transferred twice (for two gastrula generations) into eggs of the same kind, shows whether nuclei have been affected by their sojourn in foreign cytoplasm. When

nuclei are transferred to eggs of another species, the heterotypic combination (1st out-transfer) usually dies at the late blastula or early gastrula stage owing to the nucleo-cytoplasmic incompatibility. When these nuclei are back-transferred (before developmental arrest) to their own kind of cytoplasm, they again cease development at an early stage, showing that they have been affected by the foreign cytoplasm (Fischberg, Gurdon and Elsdale, 1958; Moore, 1958). The present experiments show that nuclei transferred to the egg-cytoplasm of a foreign subspecies give rise to normal development; they also show that such nuclei are entirely unchanged when transferred back to their own kind of eggs. This still holds true even when they have been exposed to foreign cytoplasm for over 60 mitoses, and also when returned to their own kind of eggs after at least 30 mitoses in foreign cytoplasm. It must be mentioned here that occasional individuals are intermediate between the nuclear and cytoplasmic subspecies for one character or another. However, these cases are very exceptional and are being investigated further.

(ii) *The effect on transplant-embryo development of the dying irradiated nucleus of* Xenopus *eggs*

Another consequence of these experiments concerns the problem of the difference between *Xenopus* and *Rana* in the potentialities of endoderm nuclei. The rate and time of onset of nuclear differentiation is about the same in *X. l. victorianus* and *X. l. laevis*—that is, there is still a small proportion of undifferentiated nuclei in the hatched tadpole gut of *Xenopus*. This is in strong contrast to the earlier onset and much faster rate of nuclear differentiation in *Rana pipiens*, in which almost no endoderm nuclei are able to develop normally after transplantation from the tail-bud stage (Briggs and King, 1957). It has been pointed out (Gurdon, Elsdale and Fischberg, 1958; Briggs and King, 1960) that this difference could be partially due to a reversal or repair of differentiation in nuclei from advanced donor stages of *Xenopus*. It is possible that hatched tadpole nuclei are all differentiated, but that some become totipotent again, after transplantation to uncleaved eggs. Totipotency might be regained if the transplanted nucleus were to absorb from the dying egg-nucleus certain substances for which it had become deficient in the course of differentiation. It is not necessary that this reversal should be an exact retracing of the steps of differentiation; it might consist only of a compensation for the differentiated state of the nucleus by the inclusion in it of other chromosome fragments. Such a process might take place in *Xenopus* and not in *Rana*, since in *Xenopus* the nucleus is allowed to die in the egg, while in *Rana* it is completely removed. If a nucleus were to take up substances or fragments from the dying egg-nucleus of another subspecies, it might be expected to assimilate some of the characters of this other subspecies. A reversal of differentiation should be most easily discovered in nuclei derived from advanced

donor stages, since these contain a greater proportion of nuclei which have lost their totipotency (Gurdon, 1960*c*). If such a reversal of differentiation does occur, it should be manifested by the expression of some " cytoplasmic " characters in transplant-embryos which were derived from hatched tadpole nuclei of one subspecies with the egg-cytoplasm of the other. The tadpoles shown in text-fig. 2 (1st and 5th columns) are derived from tadpole nuclei of *victorianus* and egg-cytoplasm of *laevis*, but in no case have they shown any *laevis* characters, either as embryos, larvae or adults. This negative result does not disprove the possibility of a reversal of differentiation, but merely constitutes some evidence against it. It confirms the belief that normal transplant-frogs derived from hatched tadpole gut cells represent entirely undifferentiated nuclei, so that the difference in rate of nuclear differentiation between *Rana* and *Xenopus* is real and not due to reversion or repair.

(iii) *The effect of serial transplantation on living nuclei*

The experiments described above provide a very critical test of whether nuclei are damaged by repeated transplantation and whether they continue to differentiate in spite of their renewed cytoplasmic environment. Since adult frogs capable of reproduction have been obtained after serial transplantation, a study of their development, growth and reproduction provides a wide opportunity for discovering in them any departure from normal. As described above, the frogs resulting from the fourth serial transfers were indistinguishable from controls in every respect including that of fertility and the development of their offspring. This shows that some nuclei at least are not in any way damaged by the technique, and that they do not undergo any additional differentiation during serial transfer. The absence of any tendency for nuclei to continue differentiation during serial transplantation provides a clear demonstration that the stimulus which initiates normal embryonic and nuclear differentiation must come in the first place from the cytoplasm. The evidence for the lack of technical damage applies not only to the somatic development brought about by transplanted nuclei, but also to the germ-line to which they give rise. This result will be of value as a control to further experiments with other more distantly related subspecies or species of *Xenopus*.

5. SUMMARY

Nuclei have been serially transplanted between two subspecies of *Xenopus laevis*. Donor nuclei were taken from embryos and tadpoles ranging in age from late blastulae to hatched tadpoles. The resulting transplant-embryos have been reared into adult frogs. These heterotypic embryos, tadpoles, and frogs show all the distinguishing characters of the subspecies which provided the nucleus, and none of those

associated with the subspecies providing the egg-cytoplasm, except for characters directly dependent on the volume of recipient eggs.

Gastrula nuclei of *X. l. victorianus* were serially transferred four times into eggs of *X. l. laevis*, and gave rise to adult frogs which were indistinguishable from typical *victorianus*, and which were also capable of normal gametogenesis giving rise to offspring of *victorianus* type. Similarly, frogs derived from *laevis* nuclei transferred for one or two gastrula generations to *victorianus* cytoplasm, and those from nuclei transferred back to *laevis* cytoplasm after a gastrula generation in *victorianus* cytoplasm, were similar to control *laevis* frogs from fertilised eggs. The conclusions are that these nuclei were entirely unchanged after more than sixty divisions in foreign cytoplasm, and were likewise unaffected when returned to their own kind of cytoplasm after replication in that of the other subspecies.

The results also show that the serial transfer of nuclei has no tendency to diminish the viability or reproductive capacity of frogs derived from them. Lastly, the fact that some nuclei from advanced donor stages are unchanged after transfer to the eggs of the foreign subspecies indicates that these nuclei were undifferentiated at the time of transfer and have not undergone any reversal of developmental potentiality, such as might occur by assimilation of products of the irradiated egg-nucleus.

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Plate IA

Photographs of selected stages in the embryonic and larval development of *victorianus*, *laevis*, and the reciprocal combinations of heterotypic transplant-embryos. Each photograph has been outlined in white, and melanophores where present have been darkened.

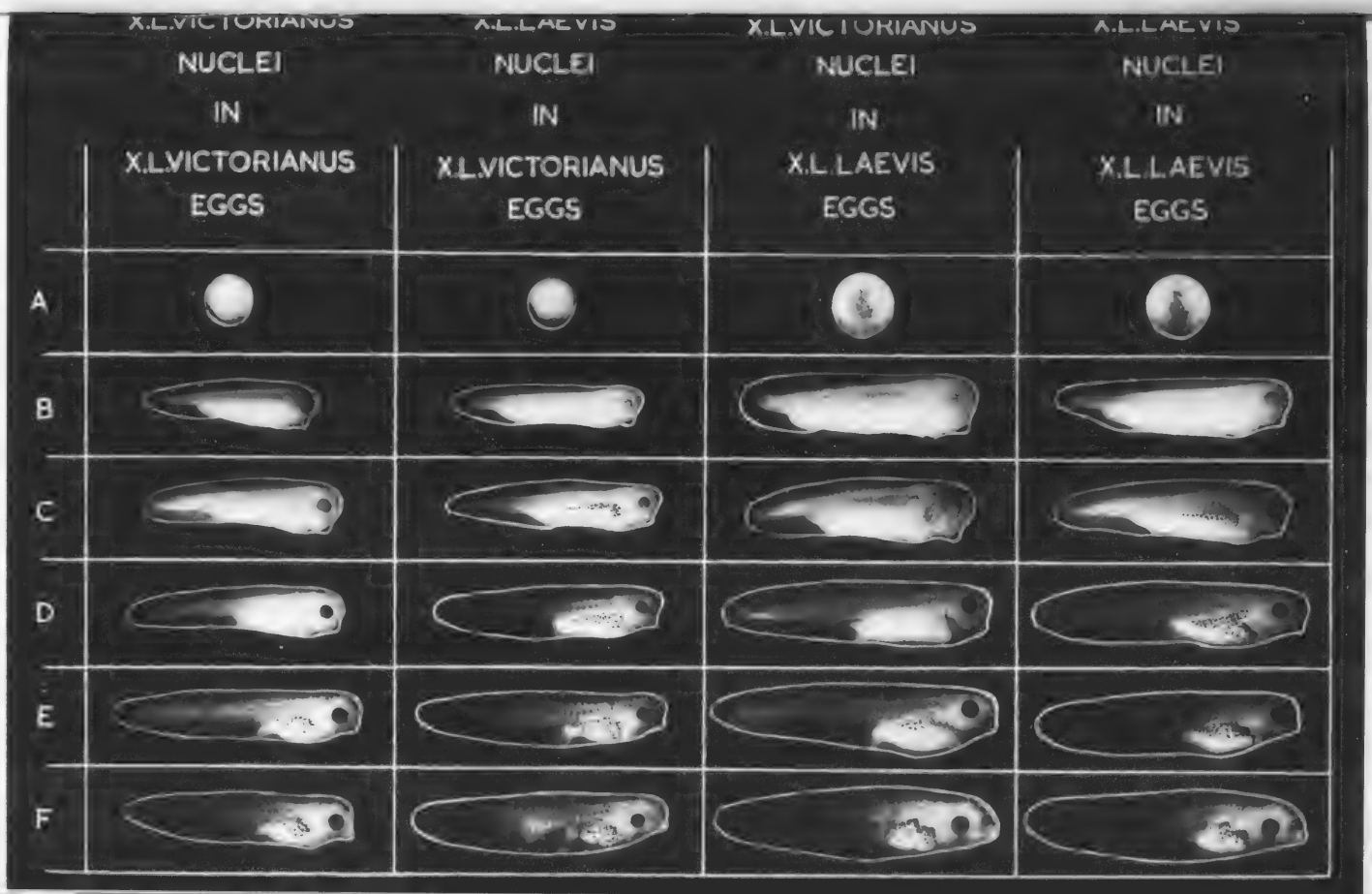
X.L.VICTORIANUS NUCLEI IN X.L.VICTORIANUS EGGS
X.L.LAEVIS NUCLEI IN X.L.VICTORIANUS EGGS
X.L.VICTORIANUS NUCLEI IN X.L.LAEVIS EGGS
X.L.LAEVIS NUCLEI IN X.L.LAEVIS EGGS
A
B
C
D
E
F

Plate IB

Photographs of adult frogs, all about one year old. The two frogs on the left are females. The remaining frogs are all males ; the 3 transplant-frogs in the lower centre are derived from nuclei transferred to *victorianus* eggs and then back to *laevis* eggs. Gastrula donors were used for all serial transfers.

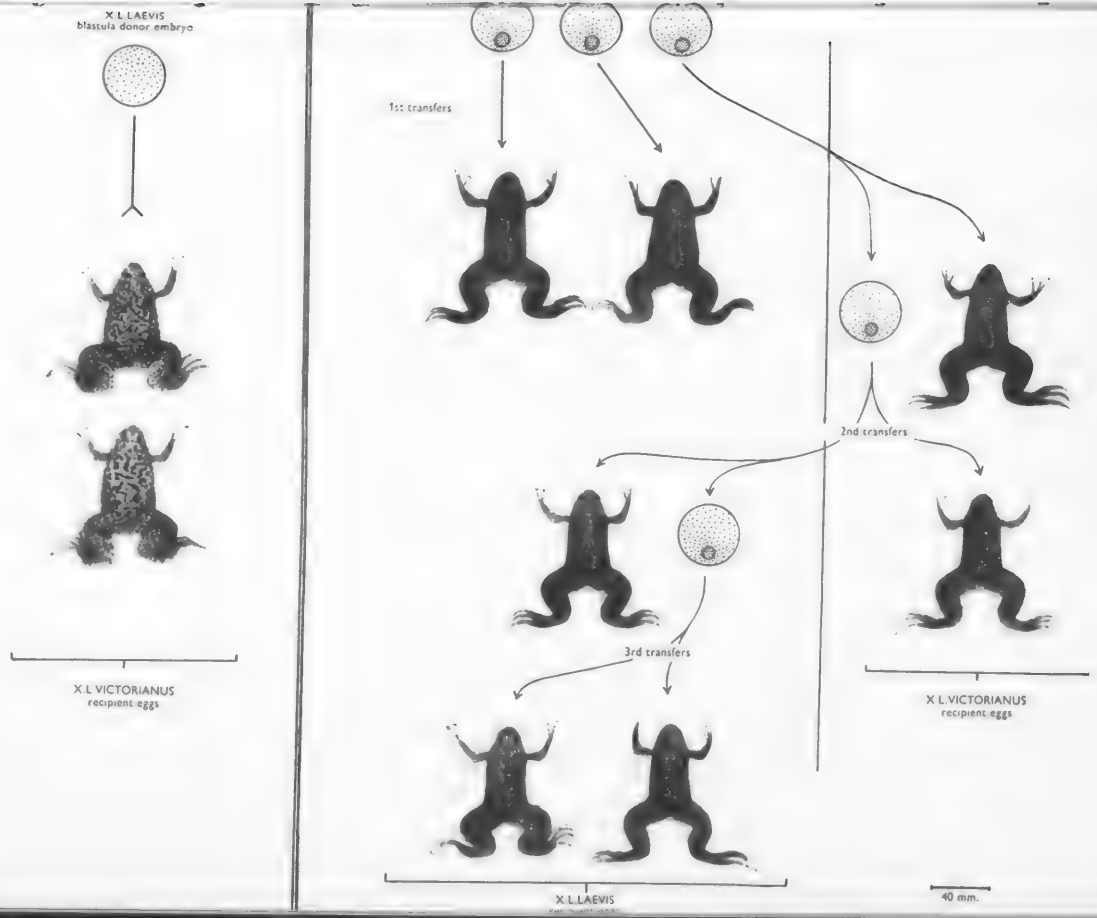
X. L. LAEVIS
blastula donor embryo
X. L. VICTORIANUS
recipient eggs
1st transfers
2nd transfers
3rd transfers
X. L. VICTORIANUS
recipient eggs
X. L. LAEVIS
40 mm.

Plate II

Photographs of adult male frogs. All have been reared under laboratory conditions, and are between one and two years old. Also all frogs are derived from *laevis* egg-cytoplasm.

LATE GASTRULA
DONOR NUCLEI
FIRST TRANSFERS
SERIAL TRANSFERS
SERIAL TRANSFERS
SERIAL TRANSFERS
CONTROL FROM
FERTILISED EGGS
X L VICTORIANUS
CONTROLS FROM
FERTILISED EGGS
40 mm

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NATURAL SELECTION IN EXPERIMENTAL POPULATIONS OF *TRIBOLIUM*

II. FACTORS AFFECTING THE FITNESS OF THE MUTANT "SPLIT" OF *TRIBOLIUM CONFUSUM* *

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1. INTRODUCTION

SEVERAL studies made in recent years have revealed a wide variety of means by which different traits may influence the fitness of an organism. Merrell and Underhill (1956), examining competition between mutant and wild type flies in experimental populations of *Drosophila melanogaster*, concluded that inequalities in the mating success of males strongly influenced the fitness of the various phenotypes. The fitness of karyotypes found in natural populations of *Drosophila persimilis* was shown by Spiess (1958) to depend upon differences in preadult viability and development rate. Using an organism other than *Drosophila*, McDonald and Peer (1960) found that in experimental populations of the flour beetle, *Tribolium confusum*, the decline in the frequency of a sex-linked lethal was due primarily to the effects of the lethal on viability and fertility of mutant males and the viability of heterozygous females. Without recourse to population experiments, other investigators have found adaptive differences in olfaction and migratory activity (Harland and Jackson, 1958) and productivity (Spofford, 1956) among various strains of *Drosophila*; and productivity has also been shown to differ between a mutant and a wild type strain of *Tribolium castaneum* (Phillips and McDonald, 1958).

With the isolation of the mutation *split* (*sp*) of *Tribolium confusum*, an opportunity arose for further exploration of the ways in which heritable differences between strains of *Tribolium* can influence fitness. This mutation depresses the reproductive capacity of the mutant strain, but precise measurements of this effect were never made (McDonald, 1959). The results reported here indicate that in experimental populations the *sp* gene is rapidly replaced by the wild type allele, and that the lower fitness of the mutant strain is due largely to the inviability, subfecundity, and impotence of the mutant females.

2. METHODS

The standard medium and environmental conditions generally employed for rearing *Tribolium confusum* were used throughout these experiments. The medium

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is a mixture consisting of 95 per cent. fine whole wheat flour and 5 per cent. dried brewer's yeast. The experiments, which fall into three groups, were conducted at a temperature of 30° C. and, with the exception of the copulation experiments, a relative humidity of approximately 60 per cent.

In the first group of experiments, populations consisting of a mixture of mutant and wild type individuals were allowed to reproduce continuously in cages operationally similar to those used for *Drosophila*. The cages have been described elsewhere (McDonald and Peer, 1960), but briefly, they are stainless steel rectangular containers about 13 cm. wide, 18·5 cm. long, and 18 cm. deep, fitted with two rows of removable compartments—eight altogether—each 4·5 cm. wide, 6·5 cm. long, and 3·3 cm. deep. These are made of perforated aluminum sheeting, and since they fit closely together the beetles can move freely from one to another. Initially, 400 grams of medium was added to each cage which filled it to about the level of the compartment rims. The food supply was renewed by removing old compartments, usually one or two each week, and replacing them with new compartments containing fresh medium. The schedule maintained in these experiments allowed each compartment to be changed once every six weeks. The medium from the old compartment was passed through a fine sieve leaving behind the adults, pupae, and larger larvae which were counted and then returned to the cage.

The second group of experiments was concerned primarily with comparing productivity of mutant and wild type individuals. In these experiments, a pair of adults (male and female) were placed in a glass vial containing three grams of medium for five days while the female deposited eggs, and then transferred to a second and third vial for two additional five-day periods. If either or both members of the pair died during the fifteen days, this was recorded, as well as the total number of adult offspring produced by each pair. Frequent transfers were employed to minimise the loss of offspring through the cannibalism of the adults.

Finally, mating activity was examined by placing a pair of adults in a small Stendor dish with an inside diameter of about 3 cm. and observing the beetles until copulation occurred. The bottom of each dish was just barely covered with medium and twenty-four dishes were observed simultaneously in a square constant temperature box about 25 cm. on edge and 5 cm. deep and covered with a sheet of clear lucite. The relative humidity ranged from 30 to 70 per cent. The female was placed in the dish first and the time elapsing between the introduction of the male and the first copulation was recorded. If copulation had not occurred by the end of forty minutes, the experiment was terminated. Since the beetles are negatively phototropic, the lighting arrangement could conceivably affect their mating activities. Therefore, in these experiments a constant source of illumination was provided by overhead fluorescent lamps, but the intensity was not measured. Finally, to eliminate the restricted activity which the insect experiences if it accidentally falls upon its back, such individuals were immediately righted.

3. RESULTS

(i) *The populations*

The mutation *split*, isolated from a wild type stock of *T. confusum* several years ago, was found to be an autosomal recessive with a high degree of penetrance. The *split* phenotype is variable, but all mutant individuals will have shortened elytra which fail to meet at the midline and expose the posterior third of the abdomen. The mutant development rate did not seem different from that of wild type, which is about twenty-eight days at 30° C., but preadult viability was found to be slightly depressed. Further, cultures of the *sp* mutant always contained an unusually large number of dead imagoes, and pair

matings involving them were frequently found to be sterile (McDonald, 1959). These preliminary observations indicated, therefore, that selection against *sp* ought to proceed rapidly and that the mutation might provide adequate material for analysing the factors determining selective values in *Tribolium* populations.

Two cages were started, each with an initial population of 462 *sp* adults and 4 wild type adults, equal numbers of each sex. The population cages showed so little activity at first that maintenance and sampling were not started until the tenth week. Then, each week

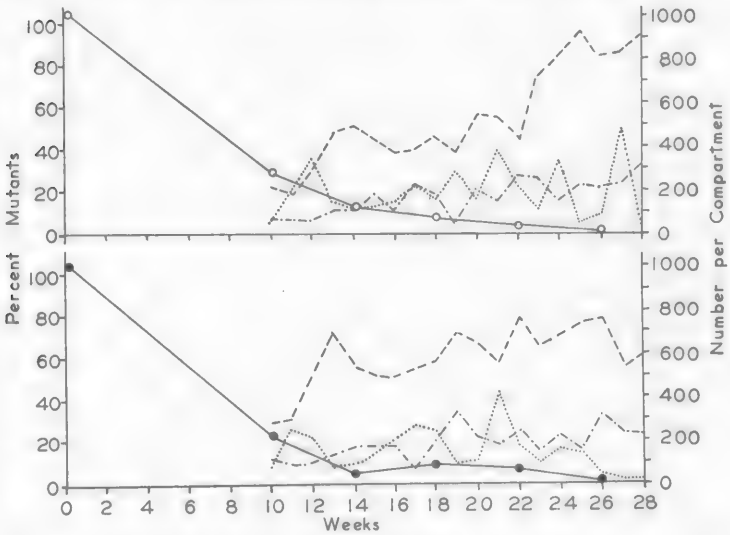

FIG. 1.—The numbers of live adults (- - -), dead adults (- . -), and pupae (. . .), and the percentage of live *sp* mutant adults (——) found in the weekly census samples from the two replicate population cages. Upper curves, Cage 21 ; lower curves, Cage 20.

the live adults, dead adults, and pupae found in the old compartment were counted, the dead adults discarded, and the living forms returned to the cage. Every fourth week a sample of 200 live adults was examined and the numbers of wild type and mutant individuals recorded. The high natality and mortality indicated by the numbers of pupae and dead imagoes found in the weekly census (fig. 1) are signs of a population undergoing rapid turnover; and, since selection was operating, were accompanied by changes in genotypic frequencies. These changes show a gradual decline in the frequency of *sp* homozygotes culminating in their elimination by the 26th week. For this reason, the cages were discontinued two weeks later.

(ii) *Productivity experiments*

Adults two days old were used to make the following crosses:

sp/*sp* ♀×+/+ ♂, 149 pair matings; +/+ ♀×*sp*/*sp* ♂, 149 pairs; +/+ ♀×+/+ ♂, 94 pairs; and *sp*/*sp* ♀×*sp*/*sp* ♂, 99 pairs. Several interesting facts emerge from the data collected and presented in tables 1, 2, 3 and 4. Table 1 shows the productivity of each kind of mating in terms of the average number of adult offspring produced per pair. Obviously, there are marked differences among all the productivities. In general, the mutant females are far less productive than the wild type females regardless of their mate's phenotype but, in addition, there is the strong indication that each type of male is

TABLE 1

Productivity of the four different crosses

Pair's Genotypes	*sp*/*sp* ♀ +/+ ♂	*sp*/*sp* ♀ *sp*/*sp* ♂	+/+ ♀ +/+ ♂	+/+ ♀ *sp*/*sp* ♂
Number of pairs	149	99	94	149
Total offspring	553	1180	7538	7441
Offspring per pair	3·7	11·9	80·2	49·9
Number of fertile pairs	67 (44·9) *	57 (57·5)	91 (96·8)	127 (85·2)
Offspring per fertile pair	8·2	20·7	82·8	58·6
	test of significance $t = 4{\cdot}0$ $P < 0{\cdot}001$		test of significance $t = 8{\cdot}7$ $P < 0{\cdot}001$	
Offspring per fertile pair per day	0·55	1·38	5·52	3·91

* Figures in parentheses are per cents.

relatively more productive with its own type of female. When mated with a wild type female, the productivity of *sp* males is little more than half that of the wild type males, but with *sp* female mates, the *sp* males' productivity is about three times greater than that of the wild type males. The same pattern of productivity persists when only fertile pairs are considered, and the statistical tests show the productivity differences between the two kinds of males with each kind of female to be significant. Table 2 reveals that the death rate among *sp* females is quite high during the first seventeen days of their adult life, for only 45·9 per cent. survive this length of time as compared to the 98·8, 97·9 and 99·2 per cent. surviving among the *sp* male, wild type female, and wild type male groups, respectively. The difference in the proportion of living and dead among the four kinds of individuals at the end of the third period is highly significant (chi

square = 411·1; $P<0.001$) with the *sp* females making by far the greatest contribution to chi square. The individuals from the *sp*/*sp* ♀×*sp*/*sp* ♂ crosses still living after the third five-day period were kept under observation for another thirty-five days. Death continued at a diminishing rate among the females for twenty-five days until only about 40 per cent. remained, whereupon no further deaths occurred. Only a few males died during this same period, so that survival never dropped below 98 per cent. Table 3 shows the number of fertile and infertile pair matings from each cross where both members of the pair lived for the entire fifteen-day period. Pairs in which either or both members had died before the end of fifteen days

TABLE 2

The number of adults surviving at the end of each five-day period

Period	Genotype							
	sp/*sp* ♀		*sp*/*sp* ♂		+/+ ♀		+/+ ♂	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
1	211	85·1	246	99·2	241	99·2	241	99·2
2	140	56·4	246	99·2	240	98·8	241	99·2
3	114	45·9	245	98·8	238	97·9	241	99·2
Initial number	248		248		243		243	

were eliminated from consideration here, so that differences between the four kinds of crosses in the proportions of fertile and sterile pairs in each would not be influenced by viability. Upon comparing the mutant female matings with matings involving wild type females, these proportions were found to be significantly different (table 3), whereas no significant difference existed between crosses involving the two kinds of males. The results indicate a much greater frequency of sterility among *sp* females than among wild type females. Finally, the data were analysed to determine if the productivity of the four crosses would be the same if only fertile and equally viable pairs were considered. This was done by selecting those pairs which were fertile the first period and lived through the entire fifteen days, and counting all the offspring produced by them during the last two five-day periods. Only seven pairs (4·7 per cent.) of the *sp*/*sp* ♀×+/+ ♂ crosses and twenty-five (25·2 per cent.) of the crosses involving *sp*/*sp* ♀×*sp*/*sp* ♂ met the above requirements as compared to 87·2 per cent. of the +/+ ♀×+/+ ♂ and 60·4 per cent. of the +/+ ♀×*sp*/*sp* ♂ crosses. These data are given in table 4 along with the mean

TABLE 3

The number of fertile and infertile pair matings where both members of the pair lived through the three periods

Pair's Genotypes	*sp/sp* ♀ +/+ ♂		+/+ ♀ +/+ ♂		+/+ ♀ *sp/sp* ♂		*sp/sp* ♀ *sp/sp* ♂	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
Fertile	31	59·2	90	96·7	125	93·2	45	73·7
Infertile	21	40·3	3	3·2	9	6·7	16	26·2
Total	52		93		134		61	

Matings compared		χ^2	P
+/+ ♀ +/+ ♂	+/+ ♀ *sp/sp* ♂	0·73	0·40
sp/sp ♀ +/+ ♂	*sp/sp* ♀ *sp/sp* ♂	1·94	0·15
+/+ ♀ *sp/sp* ♂	*sp/sp* ♀ *sp/sp* ♂	12·59	< 0·01
sp/sp ♀ +/+ ♂	+/+ ♀ +/+ ♂	30·64	< 0·01

TABLE 4

Productivity of the four different crosses where the only pairs considered were fertile during the first period and lived through the three periods

Pair's genotypes	No. of pairs	Total offspring from last two periods	Offspring per pair	Offspring per pair per day
+/+ ♀ +/+ ♂	82	5385	65·7	6·57
sp/sp ♀ +/+ ♂	7	83	11·9	1·19
+/+ ♀ *sp/sp* ♂	92	4704	50·9	5·09
sp/sp ♀ *sp/sp* ♂	25	616	24·6	2·46

of offspring per pair. Again, the most obvious difference is between the productivity of *sp* females and wild females, but there are other interesting aspects of this data which will be discussed below.

(iii) *Mating experiments*

Since the *sp* females were frequently found to be sterile and, even when fertile, less productive than wild type females, the question arose whether there might be differences in their mating behaviours as well. Pairs of mutant and wild type males and females in all possible combinations were observed by using the constant temperature box already described. Since adults frequently will not mate until

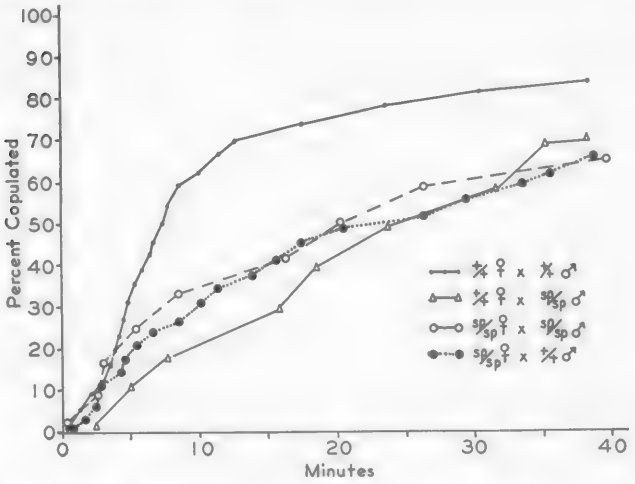

FIG. 2.—The percentage of pairs which have copulated at various intervals of time. Only the initial and final points, and approximately every fifth point between have been plotted in all curves.

three to four days old, all the insects used here were between eight and thirteen days of age. The results of the copulation experiments are presented in fig. 2 where the per cent. copulated are plotted against time. These curves demonstrate that the wild type pairs copulate much sooner than mutant pairs or either combination of mutant and wild type. Fifty per cent. of the wild type pairs have copulated within eight minutes following the introduction of the male into the dish as compared to twenty-seven minutes for 50 per cent. copulation of the +/+ ♀×*sp*/*sp* ♂ pairs and about twenty-one minutes for pairs involving *sp* females with either kind of male. The data are summarised in table 5 where the mean copulation times as well as comparisons of means and variances will be found.

4. DISCUSSION

It is clear from the results presented in fig. 1 that in the experimental populations the *sp* frequency diminishes with considerable rapidity. Since *Tribolium confusum* requires about four weeks (at 30° C.) to develop from egg to adult, a first generation was emerging between the fifth and eighth weeks, and both first and second generation individuals following the eighth week. By the tenth week, therefore, the populations were only entering the second generation, yet at this time, the frequency of *sp* individuals had decreased from 99 to approximately 25 per cent. The equilibrium frequency of *sp* individuals

TABLE 5

The time required for each of the four different crosses to achieve the first copulation. Bartlett's test was applied in the variance comparisons

Type	Pair's genotype	Total number copulated	Mean time to first copulation (minutes)	Comparison of variances	Comparison of means
1	+/+ ♀ +/+ ♂	107	8·59±0·74 var. = 58	Types 1, 2, 3 and 4 $\chi^2 = 14{\cdot}77$ $P < 0{\cdot}01$ heterogeneous	Types 1 and 4 $t = 2{\cdot}73$ $P < 0{\cdot}01$
2	*sp*/*sp* ♀ +/+ ♂	95	14·28±1·17 var. = 131		
2	+/+ ♀ *sp*/*sp* ♂	35	19·50±1·79 var. = 113	Types 2, 3 and 4 $\chi^2 = 0{\cdot}30$ $P = 0{\cdot}95$ homogeneous	Types 2, 3 and 4 $F = 4{\cdot}08$ $P < 0{\cdot}025$
4	*sp*/*sp* ♀ *sp*/*sp* ♂	39	12·42±1·72 var. = 115		

in the absence of selection would have been about 98 per cent. Obviously, the fitness of the mutant strain must be far below that of the wild type strain under the conditions provided here; and, since the relative fitness of two phenotypes must ultimately be expressed as differences in their net reproductive capacities, the census data were examined for evidence of such disparity.

The information contained in fig. 1 indicates that at the tenth week the total adult population was of the order of only 2000 individuals. This is far fewer than had been found in other *Tribolium* population cages (McDonald and Peer, 1960) where 8000 to 10,000 adults was not an unusual number at ten weeks when the populations had attained a maximum size. Furthermore, the abundance of pupae found in the weekly sample—about 150 to 190 per compartment for cages 20 and 21 respectively—indicated an expanding population, and, indeed, the adult numbers continued to increase up to the termination of the census. The reproductive capacity of the original population, which consisted almost entirely of *sp* insects, seems, therefore, to have

been severely limited resulting in a delayed population expansion. The nature of this limitation is explored in some detail below.

The results of the productivity experiments indicated that the *sp* females were primarily responsible for the lower productivity of the mutant strain. Disregarding the differences among males, the data in table 1 show that from 248 *sp* females only 1733 offspring were obtained, or about 7·0 offspring per female; whereas the comparable figure for wild type females is 61·6, roughly nine times the value for mutant females. The same calculation made for males shows them to be equal in productivity, but this is misleading for it obscures the curious differences in their productivities which are dependent to a large extent upon the type of female involved.

What follows here is an attempt to resolve productivity into its components—viability, fecundity and potency—and to assess the relative importance of these components in determining the productivity and hence the relative fitness of mutant and wild type strains.

The number of offspring produced by an insect such as *T. confusum* is subject to biological limitations imposed by the organism itself. Any particular female if provided with an adequate male will prove to be fertile or infertile; and the proportion of these two kinds of females will influence productivity of the strain. The causes of infertility are: (*a*) death—the individual dies before it can reproduce; (*b*) infecundity—it lives but produces no gametes; (*c*) impotence—it lives and is fecund, but is unable to copulate; (*d*) preadult inviability—offspring are produced, but die before reaching maturity. When the female proves to be fertile, productivity will be further influenced by: (*a*) longevity—the length of reproductive life; (*b*) fecundity—the number of eggs produced; (*c*) potency—the onset, frequency and success of copulatory activity; (*d*) preadult viability—the number of offspring reaching maturity. Productivity will also be dependent upon environmental factors such as food supply and cannibalism, but these have not been considered here.

It is apparent from table 1 that a large proportion of the mutant females are sterile, for of the 248 pairs involving *sp* females, only 124 (50 per cent.) produced offspring; whereas 218 (89·7 per cent.) of 243 wild type females were productive ($\chi^2 = 89{\cdot}7$; $P < 0{\cdot}001$). Among the males there seems to be little if any real difference of this nature since 74·5 per cent. of the *sp* males and 65·1 per cent. of the wild type males were fertile ($\chi^2 = 4{\cdot}46$; $P = 0{\cdot}04$).

The inviability of *sp* females is probably one of the most important factors contributing to the high proportion of sterile matings involving them. In view of the fact that the usual life span of *T. confusum* is five to six months (Pearl, Park and Miner, 1941), the death of over half the *sp* females (table 2) within seventeen days following eclosion is quite striking. A definite explanation for the lethal effects of *sp* cannot be given now, but Sokoloff (1960) has described a mutation,

truncated elytra, of the closely related species *Tribolium castaneum*, where the adults possess an elytral deformity similar to split and die at an early age—usually within four weeks. He suggests that water loss resulting from the exposure of the abdomen normally covered by the elytra is responsible for this inviability. Excess evaporation could indeed be involved for *T. castaneum*, like *T. confusum*, is reared in a dry flour and yeast mixture where maintenance of water balance may be a critical problem. However, this factor *per se* will not provide an adequate explanation for the inviability of split females encountered here, for the *sp* males whose elytral deformities appear equally severe showed no tendency toward early death within the fifty days they remained under observation; and, in fact, their death rate was comparable to that of normal *T. confusum*. The interesting difference in the longevity of *sp* males and females is at the moment not understood, but the sharp rise in the death of *sp* females which occurs between the first and second observation periods, when the females are seven to twelve days old, may indicate that many of them are unable to bear the physiological burden of egg production which usually reaches a maximum rate about this time. The egg, of course, contains a considerable quantity of water, so that the onset of egg laying activity may upset the water balance of the females and result in death.

Inviability cannot be the only reason for the sterility of mutant females for the data presented in table 3 clearly indicate a difference in the proportions of fertile and infertile individuals found among *sp* and wild females even where both types live equal lengths of time. Of the 113 pairs in this group containing *sp* females, 32·7 per cent. were sterile compared to 4·0 per cent. infertile pairs involving wild females; and, as mentioned previously, this difference again seems to be confined to the females only.

A viable female may still be sterile if she produces no eggs or, in other words, is infecund. The question of fecundity was explored by counting the eggs found in dissected mature females removed from mass cultures containing males. The presence of males must be considered for the fecundity of *Tribolium* females has been shown to increase markedly if they are permitted to copulate (Park, 1933) and, of course, there were males in both the cage populations and the fertility experiments.

Twenty-one females each of the mutant and wild type strains were dissected and examined under magnification. Only two of the *sp* females contained no recognisable eggs, and the remainder had from one to six eggs each in various stages of development, with a mean number of 3·5 per female. Infecundity, therefore, probably accounts for at least some of the sterile individuals found among *sp* females. None of the wild type females was without eggs and they contained from one to eighteen eggs, with a mean of 7·5 per female. This is roughly comparable to the figure found by Dick (1937) of five to

eight mature eggs per female and a variable number in different developmental stages. The mean egg numbers for the two types of females are significantly different ($t = 4{\cdot}69$; $P<0{\cdot}001$).

Another cause of the high proportion of sterile *sp* females may be the interference of the elytral deformity with mating activities. When copulating the male must position itself on the female's back, and if copulation with *sp* females is more difficult or impossible, pairs involving them would more frequently prove to be sterile. This hypothesis led to the copulation experiments where by observing the insects *in copula* the effects of the split elytra might be detectable.

The time required to achieve copulation was measured in an endeavour to quantify mating performance. The data of several experiments when pooled yield " copulation rates " whose magnitudes can be readily compared by reference to the curves in fig. 2. It should first be noted that since the *sp* female curves seem to be approaching asymptotes of approximately 70 per cent., about 30 per cent. of the *sp* females may be impotent and will never copulate at all. On the other hand, while the $+/+ \,♀ \times +/+ \,♂$ curve substantiates the finding of little infertility in these crosses (table 3), the $+/+ \,♀ \times$ *sp*/*sp* ♂ curve suggests a greater percentage of infertility than was actually found. However, the latter curve seems still to be in an ascending phase and might shortly have reached a much higher level. Obviously, these conclusions, while helpful, remain tentative until the experiments are extended over a longer period of time.

In addition to the difference in proportion of fertile and sterile *sp* and wild females there is, even when they are fertile, an inequality in their productivity. The 124 *sp* females that were fertile produced 1733 offspring or about fourteen per female as compared to the productivity of the 234 wild females who produced 14,979 offspring or 61·6 per female. If the fecundity of *sp* females is only about 47 per cent. that of wild females, the *sp* females might have been expected to produce 29 offspring per female; but since they actually produce only about half this expected value, it is necessary to seek causes other than subfecundity for the reduction in productivity.

One such cause is suggested from the fact that the death rate among fertile *sp* females is greater than among fertile wild females. This means that many of the *sp* females die before the fifteen day period is over and can no longer contribute offspring. This factor considered along with their subfecundity and the preadult inviability of *sp* offspring will almost account for the lower productivity of *sp* females. The data in table 4 show that where fertile *sp* and wild females have both lived through the three periods and were fertile at the start of this time, wild females produced 58 offspring per female during the last two periods as compared to 21·8 per *sp* female. However, the number of offspring produced by the *sp*/*sp* $♀ \times +/+ \,♂$ crosses adjusted to account for the 47 per cent. fecundity of *sp* females would be about 177; and the total offspring from the *sp*/*sp* ♀ × *sp*/*sp* ♂ crosses adjusted

for both fecundity and the preadult viability of mutant offspring of 88 per cent. would be 1489. Thus, if the effects of longevity, fecundity, and preadult viability were eliminated, the thirty-two fertile *sp* females would be expected to produce 1666 offspring or about 52 per female, which is close to but still slightly below productivity of the wild female. However, in view of the variability of the characteristics being measured this may be as good an agreement as can be expected.

Turning now to a comparison of the males, no great differences in life span (table 2) or in proportion of fertile matings (table 1) can be found, so that the productivity differences between the males cannot be attributed to these factors. For this reason, in table 4 where only fertile and equally viable individuals are compared, a pattern of productivity emerges which is similar to that found before these factors were eliminated. Furthermore, the differences in productivity of *sp* and wild males when mated with wild females is still significant ($t = 6{\cdot}1$; $P < 0{\cdot}001$) in spite of eliminating from consideration in table 4 a large number of *sp* males which were late in inseminating their females. Of the 127 fertile $+/+$ ♀$\times$*sp*/*sp* ♂ crosses, three were excluded because either the male or female died, and thirty-two (25·1 per cent.) because they were not productive until after the first period. Only nine (9·9 per cent.) of the 91 fertile $+/+$ ♀$\times$ $+/+$♂ crosses were excluded because of delayed productivity, and the proportions of delayed and immediately productive crosses in this group is significantly different ($\chi^2 = 7{\cdot}61$; $P = 0{\cdot}007$) from these same proportions in the *sp* male group.

The possibility that the males are not equally precocious—at least when mated with wild type females—is further supported by the results of the copulation experiments (fig. 2), for the copulation rates of *sp* and wild males mated with wild females are clearly seen to be dissimilar. *Sp* males, therefore, seem less adept at inseminating wild type females and, while this may be a partial explanation of the productivity differences between the males, it cannot account for the difference remaining in the data of table 4. It is conceivable that *sp* males are less fecund, or do not copulate as effectively (*i.e.*, do not transfer a sufficient supply of sperm) or as frequently as wild type males. However, these possible inadequacies would still fail to account for the apparent reversal of the productivity differential between males when mated with mutant females. These productivities (table 1) are significantly different, and in table 4 the pattern is the same although the significance is lost with the reduction in the number of pairs available for comparison.

The differences found thus far are still not sufficient to explain the precipitous drop in the frequency of *sp* in the population cages, for if the males are assumed to be equally productive and the productivity of the *sp* female is about one-sixth that of the wild female, the first generation should have been about 90 per cent. *sp* homozygotes and a second generation produced by them over 75 per cent. *sp*.

The populations at the first sampling are a mixture of first and second generations, but since the frequency of *sp* individuals is far less than 75 per cent., the depression of the productivity of the *sp* mutants apparently is augmented by the cage environment. One possible explanation may be that viabilities of mutant and wild type insects are differentially affected by the density of organisms in the cages. The number of adults and immature forms per gm. of medium is undoubtedly greater in the cages than in the vials used for the productivity experiments. There were only two adults per three grams of medium in each vial whereas in the population cages, by the tenth week, about 2000 adults occupied 400 gms. of medium, a density seven times that of the vials. Furthermore, for several weeks the average number of pupae in each cage was about 1500 indicating the presence of three times that many larvae. This is about eleven larvae per gm., which is four to five times greater than the density of larval offspring in vials of *sp* female crosses. Thus, the appreciably greater cage densities may have been partly responsible for the rapid decline of *sp* in the population. This problem and a further investigation of male productivities will be the subject of future experiments.

Finally, it is interesting to consider further the causes of the copulation rate differences found among males as well as females. Attention has already been drawn to the possibility that the elytral deformity of the *sp* female presents an obstacle to copulation. If it is assumed that both types of males have equal difficulty in overcoming this obstacle and that the wild female presents a different obstacle which is more easily overcome by wild type than by *sp* males, the results of the copulation experiments become meaningful. Unfortunately, observations of the mating behaviour of mutant and wild males with wild type females have produced no definite evidence of the existence of such an obstacle. Mating in *T. confusum* is devoid of the elaborate courtship rituals commonly found in other insects such as *Drosophila* (Spieth, 1952). The male upon encountering a female will sometimes trail after her and when she stops moving he may then attempt to mount. His efforts do not bring about any noticeable diminution of the female's activities which, in fact, may frequently lead to his being dislodged. This is the only finding which seems suggestive, for if wild type females are more vigorous than *sp* females, their movements may be more violent and apt to dislodge more easily a weak or unbalanced *sp* male.

The significantly larger variances in mean copulation time (table 5) obtained when one member of the pair is a mutant compared to the situation when both members are wild type should be noted, for if copulation is subject to interference by the effects of the *split* mutation, variation in these effects could lead to wide variation in the copulatory performance of mutant individuals. At present, all that can be said in regard to this is that the most obvious phenotypic effect of *sp*, the elytral abnormality, is highly variable in expression.

5. SUMMARY

1. In experimental populations of *Tribolium confusum*, reared in population cages, the mutation *split* (*sp*) is rapidly replaced by the normal allele, indicating that the fitness of the mutant is far below that of the wild type.

2. Responsibility for this lowered fitness of the mutant strain is not shared equally by both sexes. The productivity of mutant females is about one-sixth that of the wild type females, whereas the two types of males do not differ greatly in this respect. However, each type of male is relatively more productive with its own type of female.

3. The lower productivity of *sp* females can be traced to their inviability, subfecundity and impotence. The reasons for the male productivity differences are not clear. However, it has been found that mutant males copulate less readily and less effectively with wild type females than do wild type males, while with mutant females neither male is more effective.

4. Estimates of the *sp* frequencies expected in the experimental populations by the second generation are higher than the actual frequencies found. Possibly the greater densities of living organisms found in the cages adversely affect the mutants and intensify selection against them.

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THE MECHANISM OF HAIRINESS IN *GOSSYPIUM*

I. *GOSSYPIUM HIRSUTUM*

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1. INTRODUCTION

HAIR on the vegetative parts of the cotton plant is important. With mechanical picking the amount of trash collected with the lint is greater if the leaves are hairy and its removal is more difficult. Where the cotton jassid (*Empoasca* spp.) is a potential pest, plants lacking hair are seriously damaged. Hence in the U.S.A. glabrous plants are selected, while in India and Africa the aim in breeding work is a good cover of hair.

The cotton breeding programme of the Sudan has for some years been concerned with the breeding of jassid-resistance into Sakel cotton, which is a long staple, high quality variety of the species *G. barbadense*. Sakel is glabrescent and therefore hairiness has had to be transferred to it from other strains. Part of the transference programme included hybridization with suitably hairy strains of *G. hirsutum* which carry the desired length and density of hair; a knowledge of the genetics of hairiness within *hirsutum* was therefore desired. Data from crosses within *hirsutum* are reported here.

2. PREVIOUS WORK

Important work on the inheritance of hair in *Gossypium* is reported in a series of papers by Knight and Sadd (1954*a* and 1954*b*) and Knight (1952, 1954 and 1955). Knight's paper (1952) contains a résumé of earlier work on both genetical findings and the relationship of hair to jassid-resistance. Briefly, studies of the genetics of cotton hairiness have resulted in conflicting conclusions. Some workers report single gene control and others have found that their results called for a multigene hypothesis. In nearly all cases segregating families were classified as "glabrescent" or "hairy", or "more hairy" and "less hairy", and usually in reference to one selected part of the plant.

Knight's work in transferences of hair from *G. hirsutum*, *G. herbaceum*, *G. arboreum* and Tanguis (*G. barbadense*) to Sakel (*G. barbadense*) led him to the conclusion that all hairy types studied contained the same principal hairiness factor, to which he gave the symbol H_1. Harland (1939) investigating the Hawaiian endemic species *G. tomentosum* found a clear single factor control for hair. Simpson (1947) reported an extremely densely hairy Upland, T611, which arose by mutation,

and which also showed monogenic inheritance of the hairy character. Knight established by homology tests that the hairiness genes from *G. tomentosum* and T611 were allelic and independent of H_1; he gave the locus the symbol H_2.

Throughout his investigation of H_1 Knight was aware that this gene alone could not account for all the hairiness of plants and referred to it as a " trigger-gene " or " key-gene " so that modifiers and minor genes working with H_1 enhance density. In Sakel hairiness he examined the lower surface of the leaf and classified the plant as either " glabrescent " or " hairy ". In some of his material he found it necessary to add an " intermediate " class. Stem hairiness was not described since in most Sakels (*G. barbadense*) to which hairiness had been added the stems were glabrous or nearly so.

The distribution of hair and its density differs between strains and it is therefore important to examine the kinds of hairiness that are found before attempting to analyse the mechanism of hairiness. Balls (1912) distinguished between hair found on vegetative parts of the plant and hair found on the calyx, corolla and young boll. Harland (1939) says of an inter-Upland (*G. hirsutum*) segregation for corolla hairs, " . . . Segregation in the grade of hairiness of other parts of the plant was of the blending type, and it became evident that seed fuzz, amount of lint per seed (lint index), corolla hairiness and calyx hairiness are all part of one general hairiness mechanism ". There are many instances in the literature of a relation between the hairiness of a plant and the quality and length of lint. Zaitzev (1934) pointed out that Old World cottons show hairiness in two layers and New World cottons in one only. This two-layered character was investigated by Saunders (1959) for the Old World diploid *G. anomalum* and a single-gene suppressor was found to control the relative density of the two layers.

Further, the type of hair found may be simple and single as is found in boll hairiness (whether associated with H_1 or H_2), or stellate as found on the vegetative parts of the plant. In the New World tetraploids these stellate hairs usually have 2-4 arms whereas diploid forms often have 4-8 and one isolated form of *herbaceum* was reported as having 10-12 arms.

The Empire Cotton Growing Corporation maintains a world collection of cottons, of which 852 belong to the seven races of *G. hirsutum* (Hutchinson, 1951). These have been examined by the writer and classified for hair distribution and length. Hair distribution patterns fell into definite types: a type hairy on all vegetative parts, a type with glabrous or glabrescent laminae but hairy on stem and other vascular surfaces, a type hairy on the upper vein surfaces only and finally a completely glabrous type. The latter was found only in race *palmeri*. Out of 51 accessions of *palmeri* 49 were glabrous and the remaining 2 had only occasional hair on the vascular tissues. The combination of glabrous stem and hairy leaf is unknown for strains

of *G. hirsutum*. Yet synthetic hairy Sakels are usually of this type whether the hair has been derived from *G. hirsutum* or Tanguis (*G. barbadense*).

The other characteristics of hairiness show great variability between strains and species. Firstly, density of hair cover can range from a thick felt to a surface bearing only an occasional hair. Secondly, hair length can vary from about 2 mm. down to less than 0·1 mm. Detailed studies of the actual numbers and length of hairs per unit area of leaf were made by Parnell, King and Ruston (1949). Segregation for density and length can occur at the same time although extreme density is usually accompanied by shortness of hair.

Full understanding of all these facets of the character " hairiness " is far from being achieved. Nevertheless, clarification of any part of the complex by genetic study is an aid to breeding programmes in which hairiness is of importance.

3. METHODS

The studies presented in this paper are restricted to hairs on the lower surface of the leaf lamina, (hereinafter referred to as LLL) and on the petiole and stem (referred to as stem). Density alone is considered and the degree of hairiness is defined in terms of nine standard grades set up for the purpose and illustrated in fig. 1.

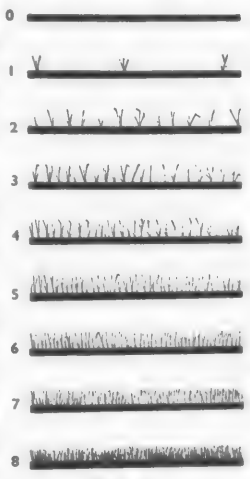

Fig. 1.

The matching of all the material against these standards was done by the same observer (the writer) and, although subjective, a satisfactory standard of consistency was maintained.

Control rows of parent hairy strains were planted generously amongst the experimental material as a check on environmental effects upon density. No density differences were observed between parental control lines.

Both LLL and stem hairiness are important for an understanding of the genetics of hairiness and the two have been presented together in correlation diagrams. A comparison of family distributions is also necessary in matching similarities or describing differences. For this reason most of the data have been converted to percentage frequencies correct to the nearest whole number, and in consequence family totals are often not one hundred exactly. The number of plants in each family is indicated with each diagram.

The grading was carried out by holding the leaf, plucked from the plant, folded in such a way that a line of lamina hairs is seen in profile. Similarly the vascular surfaces seen side-on tend to present a linear appearance. However, it must be remembered that with increase in density the numbers of hairs increase quadratically in spite of the fact that visual assessment of grade is made on a linear appearance. Therefore from about grade 3 onwards hair numbers per unit area increase enormously.

The grade of any plant is given by two figures separated by a solidus, thus 3/4 indicates an LLL grade of 3 and a stem grade of 4. The symbol 0/0 indicates no hairs on the two surfaces studied ; it does not indicate a glabrous plant, this term

being reserved for a plant entirely without hair on upper surface of leaf lamina and main vein also. The term glabrescent describes plants of grades 1 and 2. Fully hairy implies at least as hairy as the hairy parent strain.

4. DESCRIPTION OF STRAINS

Four strains of *G. hirsutum* race *latifolium* were used. Three of these carry long dense hair on all vegetative parts and are immune from jassid attack. These are: MU8b, a selection made by J. B. Hutchinson in India from the Malwa crop; Philippines Ferguson and Kapas Purao, two types from the Philippines and phenotypically indistinguishable. The fourth, UA2-20, an Acala strain from California, U.S.A., has very sparse long hair on the stem only and has no resistance to jassid attack.

T.S. 2, a strain of the race *punctatum* collected by H. E. King from the Lake Chad region of Northern Nigeria was used as the basic glabrous type since it lacks hair on all vegetative parts. It has no resistance to jassid attack.

5. RESULTS

The four hairy *latifolium* strains were crossed to the glabrous *punctatum* T.S. 2. The F_1's were graded and found to be uniform, selected plants were self-bred and F_2 families raised and graded. There were no large differences within each group of F_2 families and the data for each group were therefore bulked. Correlation diagrams of LLL and stem hair densities for the bulked F_2's are given in fig. 2. The average F_1 parent plant grade is indicated by a square on the correlation diagram.

Under field conditions MU8b can be seen to be rather more densely hairy than Ferguson or Kapas Purao. The F_1 parent plant values reflect this difference, MU8b $\times$ T.S. 2 being 3/4, and Ferguson $\times$ T.S. 2 and Kapas Purao $\times$ T.S. 2 both being 2/3. The difference in the hair density factors possessed by the three strongly hairy strains is more apparent in the F_2 distributions. Where MU8b is the hairy parent (fig. 2, diagram 1) a greater proportion of plants are in the higher density grades than in F_2's with hairy parents Ferguson and Kapas Purao (fig. 2, diagrams 2 and 3). Ferguson and Kapas Purao are almost identical in their F_2 frequency distributions and MU8b does not differ from the other two in LLL density distribution but in stem hairiness. The F_2 correlation diagram for UA2-20 $\times$ T.S. 2 (fig. 2, diagram 4) contrasts weakly and strongly hairy strains.

Knight (1952) and Knight and Sadd (1954*b*) demonstrated in hair transference to *G. barbadense* that each of the three strongly hairy strains used here contained a major hairiness gene H_1. In none of the F_2 distributions is there the discontinuity to be expected on the segregation of a major gene. It is not until the F_3 generation and backcrosses to the glabrous parent have been selfed that there is clear evidence of the segregation of a single gene of major importance.

(i) *Evidence for a major hairiness gene*

F_3 families were raised from selected plants, fully hairy, glabrescent or glabrous-leaved but with stems hairy, and finally, glabrous. It was expected that true-breeding types would be obtained from each class. Fully hairy and glabrous plants mostly provided true-breeding families but those with hair only on the stem were found to segregate giving a percentage of fully hairy plants.

In the transference of hair from *G. hirsutum* to *G. barbadense* (Sakel) true-breeding families with or without stem hair were obtained; this did not occur in the material reported here; stem hair was always present.

The self-bred progenies are set out in correlation diagrams in figs. 3, 4 and 5 for hairy parents MU8b, Ferguson and Kapas Purao respectively. As in the F_2 diagrams the hairiness grade of the parent plant of each family is given by a square at the appropriate value on

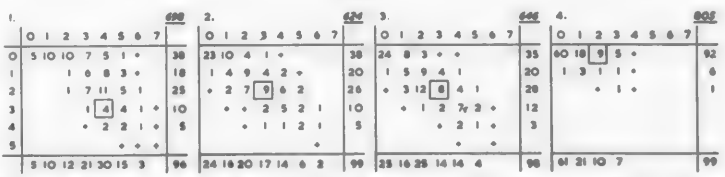

FIG. 2.—F_3 Families. 1. MU8b × T.S.2—Bulk of 5 families. 2. Ferguson × T.S.2—Bulk of 5 families. 3. Kapas Purao × T.S.2—Bulk of 5 families. 4. UA2-20 × T.S.2—Bulk of 10 families. The underlined italicised figures are bulk family totals of plants per group. + indicates less than 1% frequency.

the diagram. An examination of these families (see table 1) shows that certain of them have clear discontinuity in their distributions occurring at the glabrescent end of the scale with approximately a quarter of the plants in grade 0/0 and three-quarters in the hairy grades.

Clearly in these families a major hairiness gene is segregating since all are good approximations to a ratio of 1 glabrescent to 3 hairy expected for a dominant hairiness gene. Further in two of these families the distribution has a distinct ratio of 1 : 2 : 1. These families are G393/57, fig. 4, diagram 2 and G421/57, fig. 5, diagram 1; each is further divided at grade 4 for stem hair.

There are other families which show similar discontinuity of distribution but which do not give very good approximations to the 1 : 3 segregation. These are given below in table 2.

It will be seen that these aberrant families agree in giving ratios which approximate very closely to 1 glabrescent to 2 hairy. In the case of the MU8b data the shortage of plants in the hairy class might be ascribed to loss due to chlorophyll deficient plants since these were recorded in the F_2 families. However, it is uncertain that in MU8b chlorophyll deficiency is linked to H_1 or that this linkage in

either phase affects the segregation of hairiness (Knight, 1954). In the case of the Ferguson family G394/57 this cannot apply since no chlorophyll deficients were recorded in the cross with T.S. 2. These so-called distorted ratios may be no more than random deviations

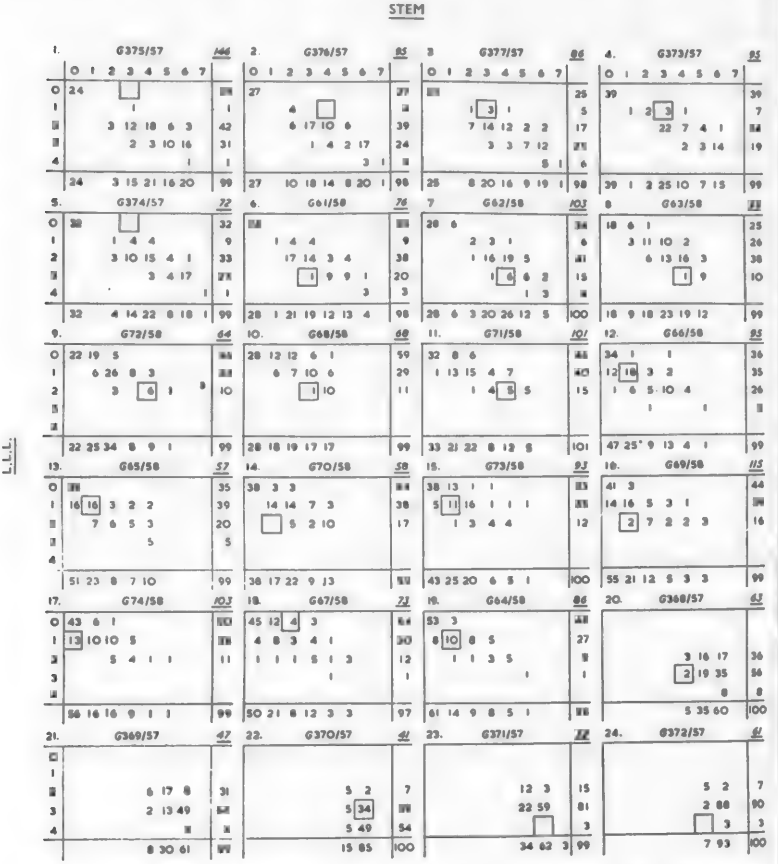

FIG. 3.—MU8b×T.S.2 F_2's (diagrams 1-5 and 20- 24). (MU8b×T.S.2)F_2×T.S.2)F_2's (diagrams 6-19).

from a single gene expectation but their uniformity argues against this and supports the hypothesis that the cause is genetic.

The Kapas Purao data provide another type of discontinuous family where the number of segregates in the glabrescent class is fewer than would be expected from a single gene segregation. These families are given in table 3.

Here again it seems unlikely that these two families coincide by

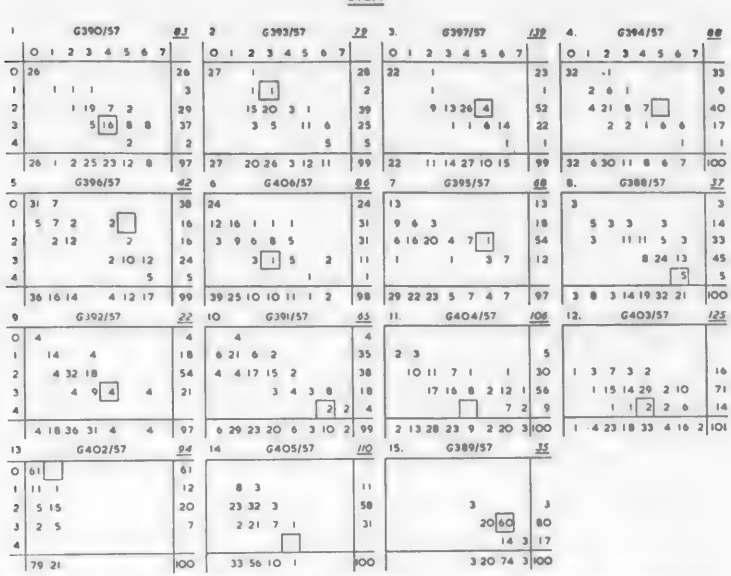

FIG. 4.—Ferguson × T.S.2 F_3 families. The underlined italicised figures are the numbers of plants per family.

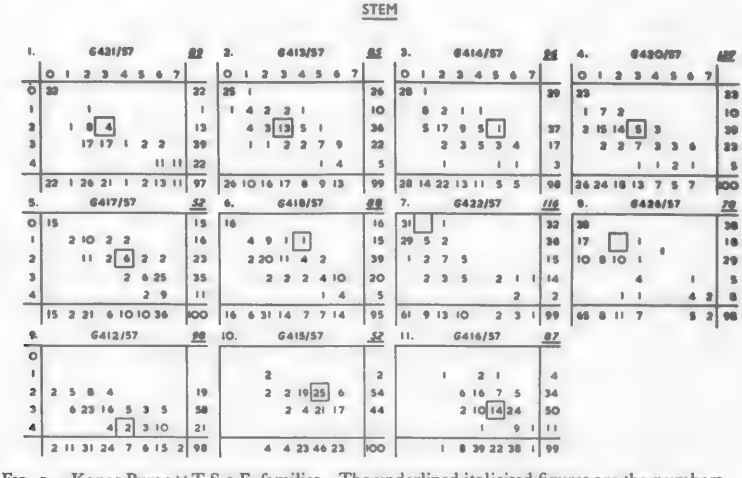

FIG. 5.—Kapas Purao × T.S.2 F_3 families. The underlined italicised figures are the numbers of plants per family.

TABLE 1

Families demonstrating the segregation of a dominant hairiness gene

	Diagram	Family	Percentage	
			Glabrescent	Hairy
Fig. 3				
MU8b × T.S. 2 F_2 . . .	1	G375/57	24	75
	2	G376/57	27	71
	3	G377/57	25	73
(MU8b × T.S. 2)F_2 × T.S. 2) F_2 .	6	G61/58	28	70
Fig. 4				
Ferguson × T.S. 2 F_2 . .	1	G390/57	26	71
	2	G393/57	27	72
	3	G397/57	22	77
Fig. 5				
Kapas Purao × T.S. 2 F_2 . .	1	G421/57	22	75

TABLE 2

Families with aberrant ratios for hairiness gene

	Diagram	Family	Percentage	
			Glabrescent	Hairy
Fig. 3				
MU8b × T.S. 2 F_2 . . .	4	G373/57	39	60
	5	G374/57	32	67
(MU8b × T.S. 2)F_2 × T.S. 2) F_2 .	7	G62/58	34	66
Fig. 4				
Ferguson × T.S. 2 F_2 . .	4	G394/58	32	68

TABLE 3

Hairiness segregation in Kapas Purao

	Diagram	Family	Percentage	
			Glabrescent	Hairy
Fig. 5				
Kapas Purao × T.S. 2 F_2 . .	1	G417/57	15	85
	2	G418/57	16	79

chance at these values widely removed from expectation. If two genes for hair initiation were segregating, one dominant and the other recessive, 19·5 per cent. of plants would appear in the glabrescent class. The observed figure is closer to this expectation than to the 25 per cent. expected for a single gene segregation. However, there is no supporting evidence for the second recessive gene. If such a gene existed it should initiate hair production when alone in the homozygous phase. It could be detected by selfing glabrous plants from the type of segregating family under discussion. If any of these plants were heterozygous for this gene their progeny should contain 3 glabrous to 1 hairy plants. In the course of this study many glabrous plants from segregating progenies derived from all three hairy parents were self-bred and in no case was such a family recorded.

Many other families occurred where segregation for hairiness was recorded over a range similar to that found in families demonstrating a discontinuity of distribution but with no similar division at any point. In the data where MU8b is the hairy parent (fig. 3, diagrams 8-19) a graded series of families of the blending type can be seen where in each subsequent family more and more plants are found at the glabrescent end of the scale. These families are all selfed progenies, the parents of which came from the backcross of the F_2 to the glabrous T.S. 2. If in the backcross to the glabrous parent dilution of factors favourable to hair production occurred this may have resulted in the telescoping of hairy segregates towards glabrescence in the subsequent self-bred generation.

Other families not conforming to the straightforward single gene pattern occur in the Ferguson and Kapas Purao material but are not strictly comparable with the MU8b families just discussed. Fig. 4 diagrams 5-12, give Ferguson × T.S. 2 F_3's and fig. 5 diagrams 7-9 give Kapas Purao × T.S. 2 F_3's. A wide range of self-bred progeny distributions is found. The Ferguson derivatives in fig. 4 are a graded series comparable with some MU8b families. G396/57 (diagram 5) is the least hairy; the families increase in hair density to G404/57 and G403/57 (diagrams 11 and 12) where the leaves have a high order of hair density, glabrescence being absent, and the main variability is on the stem alone. G396/57 (diagram 5) is unusual in that there is a clear division in stem hair distribution at grade " 3 " giving a ratio of 2 less hairy to 1 more hairy, an unexpected distribution, particularly in view of the fact that the parent plant graded 1/5. G402/57 (diagram 13) is also unusual in so far as some variation in LLL hair occurs while stem hair is almost non-existent; the parent plant was virtually glabrous at 0/1. G405/57 (diagram 14) is a uniform family which lacks high density genes as can be seen if it is compared with G389/57 (diagram 15).

The Kapas Purao derivatives are given in fig. 5. G422 and G426/57 (diagrams 7 and 8) are of interest. These two families both show telescoping of the greater proportion of plants to the lower

densities as was remarked in the MU8b data. There is, however, a difference; in the higher grades there is an isolated group of very hairy plants, comprising 6 per cent. of the population in G422/57 and 7 per cent. in G426/57. In a 15 : 1 two-gene segregation the ratio expressed as a percentage would be 93·5 : 6·5. It is apparent that two genes are segregating. They may be two hairiness genes or one hairiness gene and one modifier of large effect. G412/57 (diagram 9) is segregating for stem factors only and is strictly comparable with G404 and G403/57 (fig. 4, diagrams 11 and 12) in the Ferguson data. G415 and G416/57 (diagrams 10 and 11) may be regarded as families where the main gene is homozygous since no glabrous segregates are present, but where other modifying factors are segregating affecting the general level of density and thus giving the family a wide range of types. In the MU8b data on the other hand F_3 families G368, G369, G370 and G372/57 (fig. 3, diagrams 20-24) are fully hairy families with far less variability. Even so there are recognisable differences in density level which are consistent with the grade of the parent plants. One Ferguson F_3 of this type, *i.e.* of low variability, is G389/57 (fig. 4, diagram 15).

To summarise the evidence from the three strongly hairy *hirsutums*, all F_2 families were of a continuous type of distribution, but certain F_3 families from them gave clear monofactorial ratios, indicating the existence of major hairiness genes. The possibility of a second recessive hairiness gene was indicated in some families but no support for this was found in breeding tests; a second gene with a large effect, probably a modifier, was apparent in two families. The wide range of family type occurring makes it abundantly clear that the hairiness character is complex and that its polygenic component is of great importance.

(ii) *Modifiers*

The existence of modifiers of large effect and others of small but additive effect was inferred from the family distributions. It was decided that a direct test could be made by taking glabrous plants at random from the self-bred progenies (MU8b × T.S. 2)F_2 × T.S. 2)F_2 of plants out of the backcross families and crossing them to MU8b. A comparison of the results with the F_1 hair status of the initial cross between MU8b and T.S. 2 should reveal any difference in modifier content between the glabrous selection and T.S. 2. The data are too extensive to be fully presented in correlation diagrams and are given instead in fig. 6 as percentage frequency histograms for LLL and stem of each family. The plants may be arranged in six groups as in table 4.

Each of the glabrous selections was also self-bred and none gave hairy progeny. An examination of fig. 6 shows that no family was as good as the F_1 at grade 3/4 and all were certainly more variable. Group VI was the nearest to the F_1 standard but was no better than

grade 2 for LLL hair. The glabrous selections were therefore less favourable to hair density in hybridisation than the glabrous *punctatum* T.S. 2.

These test families could be classified as having High or Low Density levels or as segregating families. Differences within the broad classification of high or low warrant further subdivision as

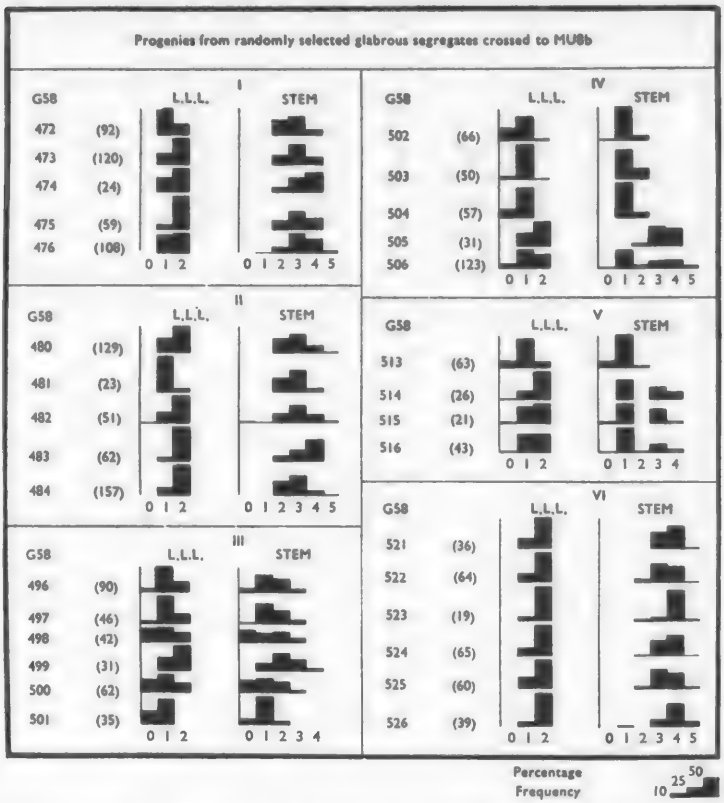

FIG. 6.—Percentage frequency histograms of hair densities of Lower Leaf Lamina and Stem of families from randomly chosen glabrous selections out of MU8b × T.S.2 derivatives backcrossed to MU8b. The number of plants per family is given in brackets.

+ or −. While these differences can be seen in the frequency histograms of fig. 6 they are more clearly apparent in family correlation diagrams which were used in the assessment but are not included here. The summary in table 5 is derived from a comparison of families.

The six density classes given above are of course arbitrary and only serve to illustrate the wide range of family type that emerged on crossing randomly chosen *glabrous* plants to MU8b. That these

differences are due to the differences between glabrous selections is demonstrated since MU8b which is common to all the backcrosses is known to be uniform with respect to hair density. In addition to this the original F_1 between MU8b and T.S. 2 was uniform and the sister F_2's raised from this were also consistent among themselves. The four families from groups IV and V showing modifier segregation

TABLE 4

Group	Families	Derivation of glabrous selections crossed to MU8b
I	G472 to G476/58	5 plants from G61/58 (fig. 3 diagram 6)
II	G480 to G484/58	5 " " G62/58 (" " 7)
III	G496 to G501/58	6 " " G64/58 (" " 19)
IV	G502 to G506/58	5 " " G65/58 (" " 13)
V	G513 to G516/58	4 " " G66/58 (" " 12)
VI	G521 to G526/58	6 " " G71/58 (" " 11)

are given in detail in table 6. True plant populations for each family are used in these correlation tables.

If grade 2 for stem is taken as the point of minimum frequency and the numbers in that class are ignored, since they cannot be allocated to either group, the ratios in table 7 are obtained.

In three families a clear indication of a single modifier is to be

TABLE 5

Classification of families in fig. 6 for hair density

Group (fig. 6)	Progeny of	Family density level						Modifier segregation
		H+	H	H−	L+	L	L−	
I	G61/58	1	3	1	.	.	.	.
II	G62/58	1	3	1	.	.	.	.
III	G64/58	.	1	.	2	1	2	.
IV	G65/58	1	.	.	.	3	.	1
V	G66/58	.	.	.	.	1	.	3
VI	G71/58	6	.	.	.	.	.	.

Glabrous selections from these families crossed to MU8*b*

H = High density level, L = Low density level } with + or −, greater or lesser density.

found and in the fourth there is the probability of two modifiers segregating. It will be noted that G516/59 does not exceed the density range of the other three families and therefore they are probably heterozygous for one modifier and homozygous for the other. These results are consistent with the density levels found in the non-segregating families.

Two important inferences can be made from these data. Firstly the glabrous *punctatum* T.S. 2 must contain modifying genes favourable

to enhanced density of hair since the F_1 of the cross MU8b × T.S. 2 is uniformly grade 3/4 whereas the progenies of glabrous selections crossed to MU8b produced variable progeny all below the F_1 standard. Secondly the main hairiness gene H_1, demonstrated by Knight (1952)

TABLE 6

Families demonstrating the segregation of modifiers

		Stem grades						
		0	1	2	3	4	5	
G506/58	LLL grades 0	1	15	.	.	.	.	16
	1	.	37	9	9	4	1	60
	2	.	7	.	13	20	7	47
		1	59	9	22	24	8	123
G514/58	LLL grades 0	.	1	.	.	.	.	1
	1	.	6	.	.	.	.	6
	2	.	6	.	8	5	.	19
		.	13	.	8	5	.	26
G515/58	LLL grades 0	.	1	.	.	.	.	1
	1	1	8	.	.	.	.	9
	2	.	2	.	8	.	.	11
		1	11	.	8	1	.	21
G516/58	LLL grades 0	.	1	.	.	.	.	1
	1	1	19	1	.	.	.	21
	2	.	8	.	9	4	.	21
		1	28	1	9	4	.	43

TABLE 7

	Observed	Calculated
G506/58	60 : 54	57 : 57 (1 : 1)
G514/58	13 : 13	13 : 13 (1 : 1)
G515/58	12 : 9	10·5 : 10·5 (1 : 1)
G516/58	29 : 13	31·5 : 10·5 (3 : 1)

to be present in MU8b, obviously depends for its full expression upon the residual genotype since in some of these families, notably group III, plants grading 1/1 and less (including 0/0) are recorded. All plants in these families must contain H_1 by virtue of the cross to MU8b. The wide range of family type undoubtedly owes its variation to the modifier content of the glabrous selections. While modifiers

of large effect have been shown to be present, the more subtle variations from family to family are no doubt due in a great measure to numerous genes of small individual effect.

(iii) *Modifiers and* H_1

Finally certain plants from the test backcrosses just examined were self-bred. These "F_2" type families can be compared with the original F_2 distribution for MU8b (fig. 2, diagram 1). The parent plants were selected as follows:

5 plants grade 2/2 or 2/2 from G496/58 } Group III, fig. 6
5 plants grade 1/1 or 2/3 from G596/58 }
5 plants grade 1/1 or 1/2 from G500/58 Group III, fig. 6
5 plants grade 2/3 from G524/58 Group VI, fig. 6

TABLE 8

Self-bred progenies of selected backcross plants of families shown in fig. 6

Family G/59	Parent plant G496/58	Parent plant grade	Percentage		No. of plants per family
			Glabrescent	Hairy	
307	.1	1/1	continuous		45
308	.2	0/1	,,		43
309	.3	1/1	,,		56
310	.4	1/1	,,		71
311	.5	1/1	,,		62
312	.6	2/2	29	71	66
313	.7	1/3	37	63	38
314	.8	2/3	29	71	61
315	.9	2/2	11	89	45
316	.10	2/2	17	83	65
	G500/58				
317	.1	1/1	17	83	73
318	.2	1/1	23	77	39
319	.3	1/1	34	66	73
320	.4	1/1	continuous		64
321	.5	1/1	19	81	64
	G524/58				
323	.1	2/3	23	77	79
324	.2	2/3	26	74	46
325	.3	2/3	25	75	56
326	.4	2/3	26	74	55
327	.5	2/3	38	62	37

The families obtained gave both continuous and discontinuous distributions. They are listed in table 8 with the percentage of plants glabrescent or hairy in those families where the two groups are distinct.

An example of each type of segregation is given in fig. 7 in percentage frequency correlation diagrams. Diagram 1 is a bulk of the data for families G307 to G311/59, all low density families with no break in the distribution, and diagram 2 is a bulk of families G312 to G316/59 all of which show a clear division in distribution. Certain

inferences can be drawn from the data of these 20 families. Firstly, they are all derived from plants heterozygous for H_1h_1 since they were taken from backcross families obtained by crossing glabrous plants to MU8b (*i.e.* $h_1h_1 \times H_1H_1$). All families are therefore segregating in respect of H_1h_1 but only a proportion of them gave evidence of this in discontinuous distributions. In those families with continuous distributions therefore the effect of H_1 is masked. A comparison of the two kinds of family show that the continuous type has the distribution of densities telescoped towards glabrescence, in particular on the stem. It seems probable that the shift in density values required to reveal the segregation of H_1 is due to modifiers of large effect such as were demonstrated in the backcross material from which these families were derived. There is some support for this in the results obtained from the 10 families derived from G496/58 and the 5 families

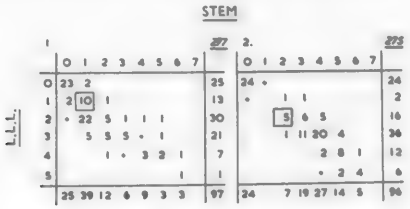

FIG. 7.—Glabrous selections out of MU8b × T.S.2 derivatives backcrossed to MU8b and selfed.

1. Bulk of *Low Density* families G307 to G311/59.
2. Bulk of *High Density* families G312 to G316/59 showing discontinuous distribution.

The underlined italicised figures are bulk family totals of plants per group. + indicates less than 1% frequency.

derived from G524/58, where the low grade parent (1/1) give a low density continuous progeny and the higher grade parent (2/2 or 2/3) gives the high density discontinuous. However, this is contradicted to some extent in the 5 families from G500/58 where 4 of them were derived from low grade parents but gave discontinuous progeny.

Amongst the families clearly segregating for a major hairiness gene it is again observed that the ratios are not always in good agreement with expectation. The last group, G323 to G327/59, has 4 out of 5 families in close agreement with an expectation of 25 per cent. of plants in the glabrescent class but the fifth has 38 per cent. of plants in this class. The remaining 9 segregating families in the other two groups vary very widely. Once again it is felt that these extreme deviations must be due to genetic causes. Evidently the expression of the major gene H_1 is very much dependent upon the residual genotype. In the extreme case the presence of the segregating genes H_1h_1 cannot be detected in low density families due to the telescoping of the density values.

(iv) *Hairiness in the absence of* H_1

It is appropriate after studying the components of hairiness in strongly hairy *hirsutums* to consider briefly the weakly hairy UA2-20. The bulk F_2 of the cross between this strain and T.S. 2 is shown in fig. 2, diagram 4. There is no evidence here of any single hairiness gene initiating hair production. Tests were also made by crossing UA2-20 to glabrous selections, known from the work with MU8b to be carrying modifiers; F_2 type families were raised. In none of these test families did the level of hairiness differ from the original F_2 nor was there any discontinuity in distribution to suggest the presence of a gene of large effect. These results suggest that either modifiers of large effect do not respond to polygenic backgrounds of low threshold value or that this type of modifier acts only in conjunction with a major hairiness gene such as H_1 which in all probability is absent from UA2-20.

6. DISCUSSION

This study of hairiness within the species *hirsutum* was intended to provide additional information concerning the mechanism of that character beyond the established presence of the key-gene H_1 (Knight, see section 2). The use of the most hairy *hirsutums* hybridised with the glabrous race *punctatum* might be expected to reveal in segregating progenies some part of this mechanism. It can be seen from the results reported here that this is complex; a wide range of family type being obtained in the F_3 and later generations. This may be contrasted with the behaviour of families segregating for the hairiness gene H_2. At the time that the hairy strains carrying H_1 were crossed to T.S. 2, Simpson's T611 carrying H_2 was also crossed to T.S. 2. The F_2 and subsequent generations showed a clear and unambiguous segregation into hairy and glabrous plants which confirmed the monogenic control of hair reported by Simpson (1947). Further the level of hair density in the presence of H_2 was always high with little or no variation.

The hairiness conferred on strains carrying H_1 is clearly different from the simple presence or absence of hair due to H_2.

The function of H_1 as a key-gene is confirmed, its presence being essential for any worthwhile cover of hairiness. The F_2 families contained a continuous range of hairy types and the segregation of H_1 was not revealed by any break in the distribution. In subsequent generations certain families clearly gave ratios of 3 hairy to 1 glabrous plants. This is attributed to a reassortment of minor genes which had previously masked the effects of H_1. It was also shown that full density levels are attained in the presence of major modifying genes acting upon H_1.

However, H_1 and its modifiers cannot initiate hair production without a suitable genetic background. This was demonstrated in plants which are known to carry H_1 in the heterozygous state but which were virtually glabrous. Conversely genes favourable to hair

production can be carried in glabrous strains but are not expressed in the absence of H_1. The use of T.S. 2 as a basic glabrous type in this study was revealing in this respect.

The part played by polygenes in the expression of the hairiness character is of great importance. It must be inferred that the general genetic background of the plant which permits the expression of H_1 is polygenic in nature. Density levels are clearly influenced to a great extent by numerous genes of small individual effect, hence the wide variety of family types as is seen in fig. 6 and tabulated in table 5. Some polygenes are also specific since in some cases the LLL is influenced while the stem is not and in other cases the stem densities are more variable than the LLL. The multigene nature of the H_1 hairiness complex clearly could confer great plasticity to that character.

These data on the mechanism of hairiness are of considerable importance for breeding work in circumstances where hairiness is a desirable characteristic of the crop. Especially is this so where hairiness has to be transferred onto a glabrescent commercial type from a hairy donor. It is quite clear that in order to breed a cotton with a dense enough cover of hair to achieve resistance to jassid many genes concerned with hair production must be brought together. These genes decide the distribution, length and density of hair. This might be done more simply by using H_2, which is a stronger gene than H_1; however, H_2 is believed by Simpson (1947) to be associated with short lint, and would therefore be undesirable. Dark (1960) reporting upon H_2 in Sakel (*G. barbadense*) found that this association could be broken, in which case this gene could be of value. However, there is less flexibility in the hairiness character as determined by H_2.

In this study on the species *hirsutum* LLL hair and stem hair were nearly always strongly correlated and no plant was produced strongly hairy on the stem and not on the leaf or vice versa. In *G. barbadense* on the other hand strains with hair confined to the leaf have been bred following gene transferences from *hirsutum*. Studies on the components of hairiness distribution in these interspecific transferences will be described in a later paper.

7. SUMMARY

Densely hairy strains of *G. hirsutum* race *latifolium* were hybridised with a glabrous strain of race *punctatum*. From segregating progenies certain conclusions were drawn concerning the mechanism of hairiness within the species. It was clear that hairiness is a complex multigenic character with both major and minor genes concerned. In this study, only hair density was examined though length and distribution are equally important components which can vary independently of density. Certain types of gene action were noted:

(i) H_1 is a hairiness gene of large effect which under favourable

genotypic conditions greatly adds to the general level of hair density.

(ii) There are modifying genes, both major and minor, active only in the presence of H_1 but capable of effecting considerable change in density level.

(iii) Low level hair density due to minor hairiness genes (*e.g.* UA2-20) occurs in the absence of H_1. The level of hairiness of this complex is not raised by the addition of genes from (ii).

A search was made for any additional major hairiness genes other than H_1 but none was found. The role of H_1 in the hairiness complex was confirmed as that of a key-gene and the understanding of the part it plays was extended.

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NOTES AND COMMENTS

CORRELATIONS BETWEEN CHAETA NUMBER AND FLY SIZE IN *DROSOPHILA MELANOGASTER*

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Sternopleural chaeta number in *Drosophila melanogaster* has been used in several extensive experiments designed to explore the effects of natural and artificial selection, and to throw light on the genetic constitution of natural populations. It is, therefore, relevant to ascertain the developmental and physiological factors influencing sternopleural chaeta number, and to find out the function of the chaetæ and how this may vary with number.

The function of these chaetæ is little understood and must await further experimentation. However, factors influencing chaeta number are more easily suggested. Three that seem likely to be of importance locally in the area in which chaeta producing initial cells differentiate are (1) the strengths of the inhibition fields around a chaeta initial (Stern, 1954), (2) the reactivity of cells to the inhibiting factors, and (3) the area of the sternopleural region. Naturally, any of these could be influenced by other factors, and the whole situation must be exceedingly complex.

The easiest of these factors to assess is the size of the fly, as measured by weight, and its effect on the area of the sternopleural region, The latter will be a function of cell size and number, and consequently is likely to depend on factors influencing fly size. Temperature and degree of crowding are known to affect fly size. At high temperatures and high crowding fewer chaetae are present than at low temperatures and low crowding (Mann, 1923 ; Plunkett, 1926). Recent data of Beardmore (see Thoday (1958) and Beardmore (1960)) also demonstrate a fall in chaeta number with increasing temperature.

Experimental results we have obtained, largely independently of each other, have converged to demonstrate a rather general correlation between chaeta number and fly size. For example, it has been shown that as temperature is increased, flies become smaller and have fewer chaetae (table 1). Similarly, under high levels of competition, flies are smaller and have fewer chaetae, and the same effect (fig. 1) occurred when phenyl-thio-carbamide (P.T.C.) was added to the medium. P.T.C. was shown by Parsons and Kroman (1961) to reduce fly size, but to have no other obvious morphological effect.

Now, it seemed reasonable, on the basis of these observations to suspect that selection for increased or decreased chaeta number might produce correlated responses of fly size. This suspicion was confirmed (table 2) using a chaeta number reducing second chromosome produced by Gibson and Thoday (1959) and Thoday and Boam (1959) in a chaeta number selection experiment. Table 3 shows that in flies produced by selection for high chaeta number by Thoday and Boam (1961), there is a correlation between chaeta number, sternopleural plate area and coxa length.

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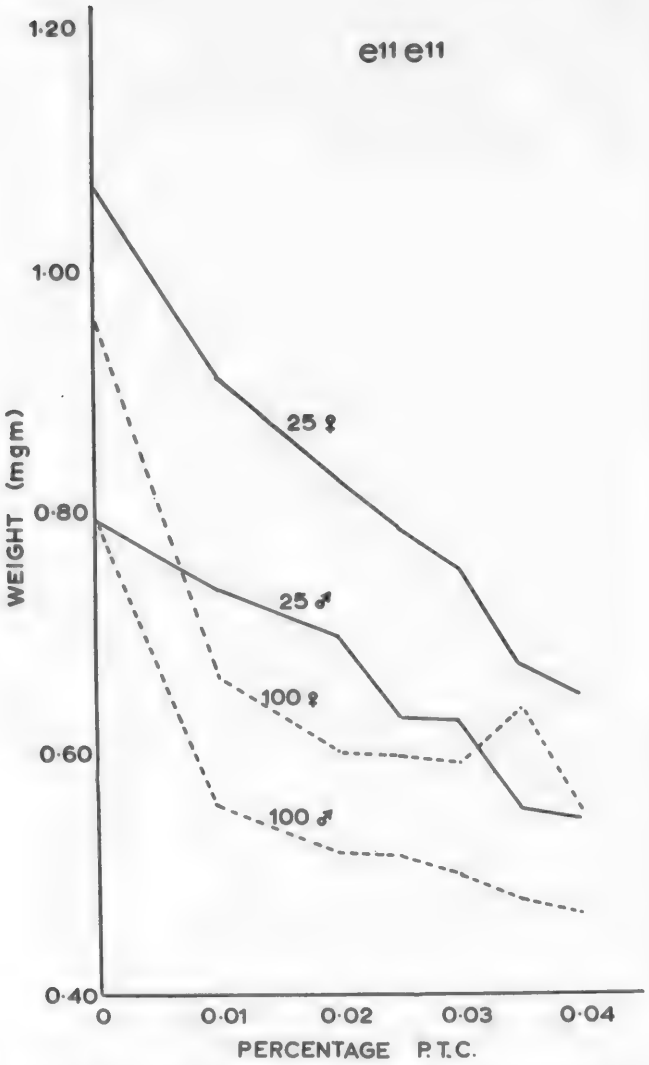

FIG. 1a.—Mean fly weight of ebony ($e^{11}e^{11}$) flies at different concentrations of P.T.C. at two levels of competition, namely 25 and 100 larvae per replicate.

Weissman's ring glands have been grafted from high chaeta number genotypes to low, and this led to an increase of both weight and chaeta number. This experiment needs to be elaborated and extended before much significance can be attached to the result; but it shows that some chaeta number genes act by influencing the hormone balance of

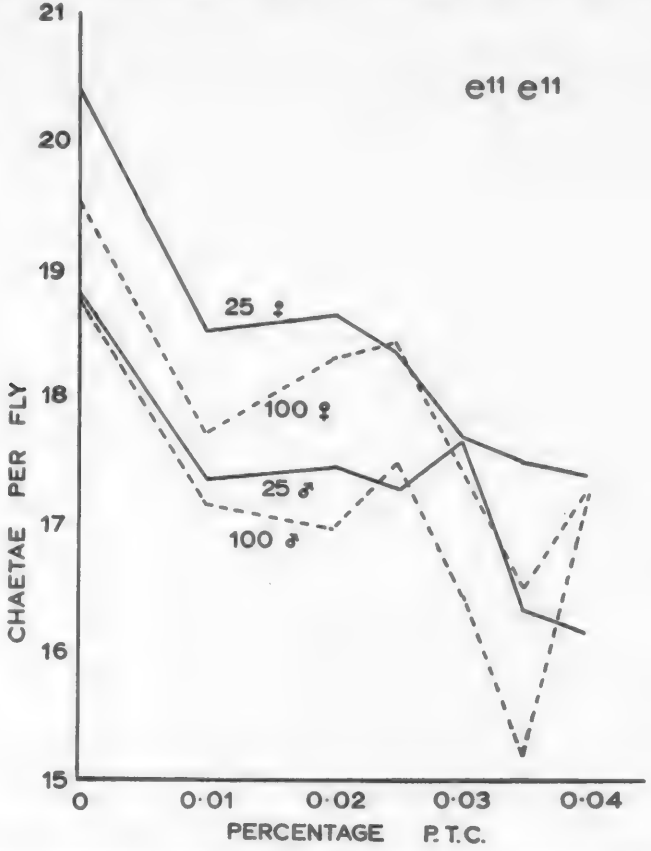

FIG. 1*b*.—Mean chaeta number of ebony ($e^{11}e^{11}$) flies at different concentrations of P.T.C. at two levels of competition, namely 25 and 100 larvae per replicate.

the fly. It is also supposed that P.T.C. may influence fly size by some complex interaction with hormone production (Parsons, 1961).

We conclude, therefore, that fly size and chaeta number are generally correlated. In a selection experiment the correlation may break down somewhat, since chaeta number is being directly selected, whereas fly size is only being indirectly selected through selection for chaeta number.

Chaeta number has been suggested to be a trivial character by Reeve (1960). This seems unlikely on general grounds, since chaeta production

TABLE 1

A comparison of homozygous y bw st *flies at different temperatures*

	Emergence time (days)		Mean chaeta number per fly		Body weight (mgm.)	
	♀	♂	♀	♂	♀	♂
20° C.	14·83	14·25	19·28	18·55	1·017	0·863
25° C.	8·64	8·47	18·52	17·68	0·953	0·801
28° C.	6·25	6·12	17·83	17·55	0·756	0·523

Each entry in the table represents the mean value of 200 flies.

TABLE 2

A comparison of a low chaeta number and a standard chromosome at 25° C. and 28° C.

	$\frac{bw}{bw}\frac{st}{st}$				$\frac{L^*}{bw}\frac{st}{st}$				Effect of L* on chaetae per fly relative to *bw/bw*
	Mean chaeta no. per fly		Body weight (mgm.)		Mean chaeta no. per fly		Body weight (mgm.)		
	♀	♂	♀	♂	♀	♂	♀	♂	
28° C.	17·76	17·40	0·728	0·653	15·88	15·73	0·620	0·495	−1·78
25° C.	18·54	17·84	0·964	0·808	16·12	15·51	0·854	0·673	−2·37

Each entry in the table represents the mean value of 100 flies.
* L = Low chaeta number second chromosome.

TABLE 3

Correlation between sternopleural chaeta number, sternopleural plate area, length of the coxa of the second leg, and the asymmetry of these variables

Line	Sternopleural chaeta number		Sternopleural plate area ($mm^2 \times 10^5$)		Length of coxa of 2nd leg (mm.)	
	Mean	Mean asymmetry *	Mean	Mean correlated † asymmetry	Mean	Mean † correlated asymmetry
Selected Line 1 .	22·52	2·00	154·00	8·32	0·145	0·021
Selected Line 2 .	32·89	3·70	188·80	9·95	0·160	0·013
Selected Line 3 .	37·21	2·42	205·44	7·71	0·163	0·011

* Asymmetry measured by taking the mean of the sides with the highest chaeta number and subtracting that of the sides with the lowest chaeta number.
† Asymmetry measured by taking the mean of the areas of the sides with the highest chaeta number and subtracting that of the sides with the lowest chaeta number.
Each entry in the table is based on the mean values for 20 female flies.

must involve metabolic effort, and some chaetæ are sensory organs which indicates that their pattern and number must be of some significance. In wild flies, patterns and number are rather constant, though they can be altered by artificial selection, which implies stabilising selection under natural selection. Beardmore (1960) has shown that natural selection influences chaeta number and asymmetry, thus showing the relevance of the character to fitness.

Furthermore, we have evidence that correlated with chaeta number asymmetry, is asymmetry of sternopleural plate area and perhaps the length of the coxa of the second leg (table 3). Hence asymmetry of chaeta number can

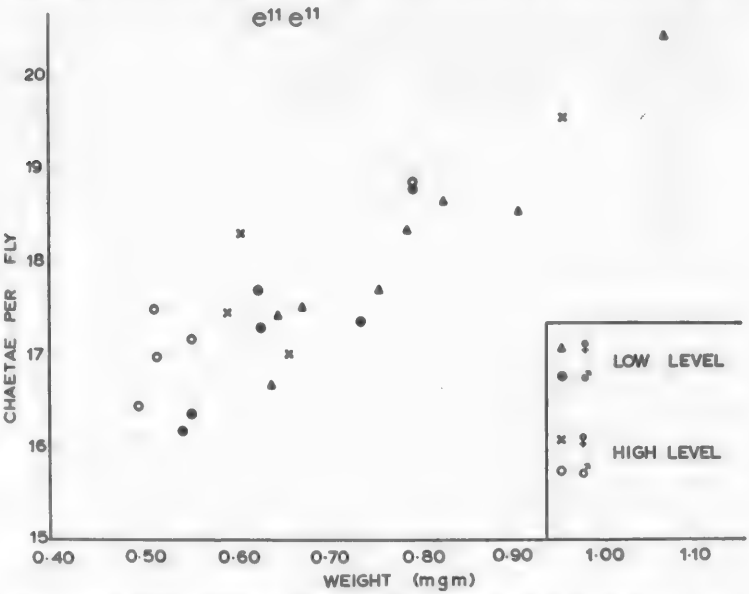

FIG. 1c.—The relation between fly weight and chaeta number for ebony ($e^{11}e^{11}$) flies. The low level represents 25 larvae per replicate, and the high level 100 larvae per replicate.

be regarded as a measure of developmental stability, relevant to more than number itself, which is perhaps why it is a character of some adaptive significance. It should be pointed out here that a measure is useful for assessing adaptive significance, whether adaptive significance arises from the character measured, or from some partly or wholly correlated character.

In conclusion, it must be stressed that chaeta number is a complex character, subject to natural selection, and that many variables independent of fly size are likely to affect chaeta number. Some of these variables are being investigated at present. Until much more is known about this character, the relative importance of the correlation with size and of other aspects of chaeta number associated with fitness will be impossible to assess.

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SEGREGATION IN TETRAPLOID BLACKBERRIES

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1. INTRODUCTION

Modern blackberry breeding is largely confined to improvements at the tetraploid level, and is particularly concerned with the production of early thornless types. The thornless character is controlled by a recessive gene which was originally given the symbol *A*/*a* by Crane and Darlington (1932); but following the usage by Lewis (1939) of *S*/*s* (= spineless) for the character in *Rubus idaeus* this latter symbol has now been generally adopted.

This character is particularly convenient for a study of tetraploid segregation in F_2 progeny, since differences between the alleles *S* and *s* can be readily distinguished during the cotyledon stage of recently emerged seedlings. At this stage, the *S* plants have glands along the edges of the cotyledons, whereas the homozygous recessive *s* plants possess naked cotyledons. The amount of mis-classification is very low, being confined largely to seedlings with abnormal cotyledons and to very late germinators. Large populations can, therefore, be raised and classified.

Darlington and Crane (1932) examined the F_2 progeny from a cross

between *R. rusticanus* (var. *inermis*)—with an unreduced egg cell ($n = 14$)—and *R. thyrsiger* ($2n = 28$) for segregation of the thornless character. Their results, based on a total segregating population of 872 seedlings, gave ratios similar to that expected for so-called " chromatid " segregation (20·8 : 1) instead of the 35 : 1 predicted if " chromosome " segregation was operating.

In view of the relative ease with which large F_2 populations could be raised, it was decided to carry out a series of crosses designed to determine not only the F_2 ratios, but also to see whether these ratios varied with the parents used.

Throughout these experiments the commercial tetraploid variety Merton Thornless ($2n = 28$), which is nulliplex for the recessive *s* gene, was used as the female parent. The three cultivated tetraploid thorned blackberries used as the pollen parents were Himalaya Giant (*R. procerus*), Edward Langley (*R. calvatus*) and Cut-Leaf (*R. laciniatus*). These three species are almost certainly *SSSS* in constitution, as F_1 progenies with Merton Thornless were all thorned.

2. ANALYSIS AND RESULTS

A preliminary analysis of the F_2 ratios obtained for the thornless gene in the three crosses showed significant total deviations from both a 35 : 1 and a 20·8 : 1 ratio. In order to test the consistency of the segregations within and between crosses (groups) it was necessary therefore to use the test devised by Brandt and Snedecor (see Mather, 1951). The resulting χ^2 values clearly showed that heterogeneity was present both within and between groups (table 1). By considering each of the three groups individually, the heterogeneity within groups was shown to be mainly attributable to the cross involving *R. laciniatus* (table 2). Whilst the omission of families 82 and 88 satisfactorily removed the heterogeneity from within this group, there was still heterogeneity between groups at the 5 per cent. level of significance. Inspection of table 3 indicates that the heterogeneity between groups is due chiefly to differences between *R. calvatus* and *R. procerus* with respect to the *S : s* ratio, since *R. laciniatus* has an intermediate value.

After omitting families 82 and 88, it was found that overall there was no longer a significant deviation from a 20·8 : 1 ratio, although the cross involving *R. calvatus* did in fact differ from such an expectation at the 5 per cent. level of significance (table 4). The production of more thornless individuals than expected on the basis of a 35 : 1 ratio can only be due to a higher frequency of formation of recessive gametes, which in turn will depend on two factors ; the mean equational separation of the locus under consideration and the non-disjunction of adjacent chromosomes. The product of these, which estimates the amount of double reduction (a), can be calculated from the expectation of each of the two classes (see Mather, 1936 ; Fisher and Mather, 1943). Higher values of a indicate that recessive gametes were produced more often, resulting in the formation of a larger number of thornless individuals (table 3).

Turning to the two anomalous families, 82 and 88, both of which showed such a marked deficiency of thornless plants, the values of a obtained for each family separately were negative and therefore meaningless. These two families would seem to pose a different problem from the remainder and therefore can be legitimately omitted from the present consideration.

TABLE 1

Tetraploid segregations in Rubus

Merton Thornless × *R. procerus*			Merton-Thornless × *R. calvatus*			Merton Thornless × *R. laciniatus*		
Family no.	No. of *s* plants	Total	Family no.	No. of *s* plants	Total	Family no.	No. of *s* plants	Total
59	14	417	75	1	77	82	1	228
60	24	622	77	2	121	88	6	475
66	13	328	69	6	213	80	2	98
63	13	315	73	4	138	54	13	504
62	11	230	72	29	987	84	7	253
64	4	83	74	8	269	79	16	497
67	4	82	71	3	88	86	8	242
52	9	172	68	4	87	78	11	332
65	19	352	76	25	455	85	8	201
53	14	255	56	18	298	55	17	371
57	12	197	...	...	...	83	9	173
61	7	113	...	...	...	87	20	316
51	2	30	...	...	...	81	16	228
58	19	248	...	...	...	...	...	...
Total	165	3444	...	100	2733	...	134	3918

Grand totals . . .	399	10,095
	Within groups	Between groups
χ^2	56·62	9·93
Degrees of freedom .	34	2
Probability . .	0·01-0·001	0·01-0·001

TABLE 2

Test of within group heterogeneity for the 3 F_2 progenies

Group	χ^2	Degrees of freedom	Probability
R. procerus	10·42	13	0·7—0·5
R. calvatus	14·44	9	0·2—0·1
R. laciniatus	35·28	12	< 0·001
R. laciniatus (with families 82 and 88 omitted)	17·17	10	0·1—0·05

TABLE 3

Values of α for the 3 F_2 progenies

Group	Ratio *S* : *s*	α
R. procerus	19·87 : 1	0·1565±0·0249
R. calvatus	26·33 : 1	0·0735±0·0282
R. laciniatus (except families 82 and 88) .	24·31 : 1	0·0962±0·0259

A comparison of the values of α obtained in this experiment with those obtained by Catcheside (1956, 1959) showed them to be intermediate between the sugary-1 locus in tetraploid maize at the lower end and the *r* locus in tomato at the upper end of the range.

3. CONCLUSIONS

If families 82 and 88 are omitted, the results of these experiments support the earlier work of Crane and Darlington in so far as the ratios obtained approximate more closely to the 20·8 : 1 predicted on the basis of " chromatid segregation ".

Taken as a whole the F_2 progenies are homogeneous within crosses, provided families 82 and 88 are excluded. The reasons for these two families being so deficient in thornless individuals cannot be determined exactly at present, but it could be attributable to inviability, linkage to a lethal, or visual mis-classification due to the dark colour of *R. laciniatus* and its hybrids masking the manifestation of the glands on the cotyledons.

TABLE 4

χ^2 analysis for a 20·8 : 1 ratio after omission of families 82 and 88

(*a*) Item	D.F.	χ^2	P.
R. procerus . . .	1	0·33	0·70—0·50
R. calvatus . . .	1	5·37	0·05—0·02
R. laciniatus . .	1	2·99	0·10—0·05
Total . .	3	8·69	...
(*b*) Overall ratio .	1	3·67	0·10—0·05
Heterogencity .	2	5·02	0·10—0·05
Total . .	3	8·69	...

The fact that the *R. calvatus* and *R. laciniatus* groups (except families 82 and 88) have similar values of α clearly indicates that the meiotic behaviour of the chromosomes is the same in these two crosses, at least as far as the present data go. The *R. procerus* group, on the other hand, gives a significantly higher value for α. This can mean either that chiasmata form more often between the centromere and the *s* gene, or multivalent formation is more regular and more nearly complete in this cross. Discrimination between these two alternatives, however, rests ultimately on cytological analysis. If the former proves to be correct then genetic control of the distribution and frequency of chiasma will have been demonstrated. Genotypic control of chiasma distribution and frequency has been found by Rees (1955) in rye. He was able to show that significant differences existed between various inbred lines, but not generally between sub-lines within these lines. However, the demonstration of genotypic control in *Rubus* depends on the assumption that for all species the physical distance between the *s* gene and the centromere is the same ; an assumption which will hold only in the absence of an inversion carrying the *s* gene.

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GENIC CONTROL OF ANEUPLOIDY IN *PENNISETUM*

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1. INTRODUCTION

Perennial *Pennisetum dubium* is an irregular high polyploid in which chromosome numbers vary from 14 to 84 in root tips, 47 to 74 at MI and 9 to 37 at MII. This variation, which occurs from cell to cell in the same root tip or anther, has been shown to be the result of various mitotic and meiotic abnormalities which are under genetic control. The single perennial hybrid plant, produced from crosses between this species and the regular annual diploid *P. typhoides* ($2n = 14$), has only 21 chromosomes (Gildenhuys and Brix, 1958).

The mitotic behaviour of the 21 chromosomes of this sterile hybrid is nearly normal, but when attempts were made to double the complement by colchicine treatment, abnormalities occurred which give further evidence of genetic control of aneuploidy.

Although many different techniques were employed in attempting to double the chromosome number of the hybrid, none was successful. This was unexpected, since neither Krishnaswamy and Raman (1954) nor ourselves, using different techniques, had great difficulty in obtaining amphidiploids from the hybrid *P. typhoides* ($2n = 14$) $\times$ *P. purpureum* ($2n = 28$). When it became evident that the hybrid between *P. typhoides* and *P. dubium* was reacting differently to colchicine from that between *P. typhoides* and *P. purpureum*, identical treatments were applied to each hybrid to try to determine the nature of the difference between the two.

2. METHODS

Two or three cuttings, each consisting of two to three nodes, were taken from the lower part of mature tillers. These cuttings were totally immersed in a 0·01 per cent. aqueous solution of colchicine for 3 days and then washed and placed in a

nutrient solution. All the root tips which developed at the nodes were removed and fixed every 24 hours over a period of 16 days, after which the cuttings which were still alive were planted in the field. When the cuttings had grown in the field for one year, chromosome counts were again made in the root tips. Because of the failure to double the chromosome number of the hybrid *P. typhoides* × *P. dubium*, cuttings were taken after one year from the previously treated plants of this hybrid and subjected to exactly the same treatment as the original cuttings. Altogether over 100 cuttings of *P. typhoides* × *P. dubium* and over 50 cuttings of the *P. typhoides* × *P. purpureum* hybrid were treated and of these 26 per cent. and 32 per cent. respectively survived.

Chromosome counts were recorded only for intact cells in which there could be no doubt as to the exact number of chromosomes present.

3. OBSERVATIONS

Before treatment no mitotic abnormalities were found in the hybrid *P. typhoides* × *P. purpureum* ($2n = 21$), while in *P. typhoides* × *P. dubium* only 2 cells out of 153 had chromosome numbers differing from 21—these two cells had 24 and 25 chromosomes respectively.

After treatment, most of the root tips of *P. typhoides* × *P. purpureum* had either 21 or 42 chromosomes, but sometimes there was a mixture of 21 and 42, or numbers in the neighbourhood of 84. After one year, the surviving plants either had 21 or 42 chromosomes, indicating that the mixoploids had stabilised at either 21 or 42.

In *P. typhoides* × *P. dubium* on the other hand, the effect of colchicine was quite different. Not only did a wide variation in chromosome number occur from cell to cell, but abnormalities similar to those observed in the polyploid parent (Gildenhuys and Brix, 1958) made their appearance.

In the first 16 days after treatment chromosome numbers varied from 14 to over 42, even in the same root, but, because of the difficulty of making accurate counts, numbers higher than 42 were not recorded. These higher numbers were, however, not always in the neighbourhood of 84, as might have been expected. The most frequent number was 21, which occurred in 32 per cent. of the 47 cells examined (fig. 1A). One year later, the range had narrowed down to 15-25, and by then the vast majority of cells (88 per cent.) contained 21 chromosomes (fig. 1B).

The results of the second treatment were similar to those of the first. Counts made for 16 days after treatment showed that the range of chromosome numbers had again increased to 14 to over 42, with 21 again the most frequent number (27 per cent. of 130 cells—fig. 1C). Four months later this range had narrowed down to 14-25 and of the 59 cells counted 66 per cent. had 21 chromosomes.

After both treatments, tripolar, quadripolar and split spindles, groups of chromosomes, etc., such as have been described in detail (Gildenhuys and Brix, 1958) were observed, but with a much lower frequency than in the polyploid parent. These abnormalities may lead to chromosome elimination and they probably account for the aneuploidy following colchicine treatment.

4. DISCUSSION

The two perennial hybrids, whilst similar in their mitotic behaviour, react in a strikingly different manner to treatment with colchicine. They have a common female parent in the regular annual diploid *P. typhoides*

and have therefore the same cytoplasm. The male parents are, however, very different. *P. purpureum* is a regular tetraploid and the hybrid with *P. typhoides* behaves as a regular amphidiploid when the chromosome complement is doubled (Krishnaswamy and Raman, 1954). *P. dubium*, on the other hand, is an irregular high polyploid which, although a facultative aposporic apomict (Gildenhuys and Brix, 1959), can produce fertile

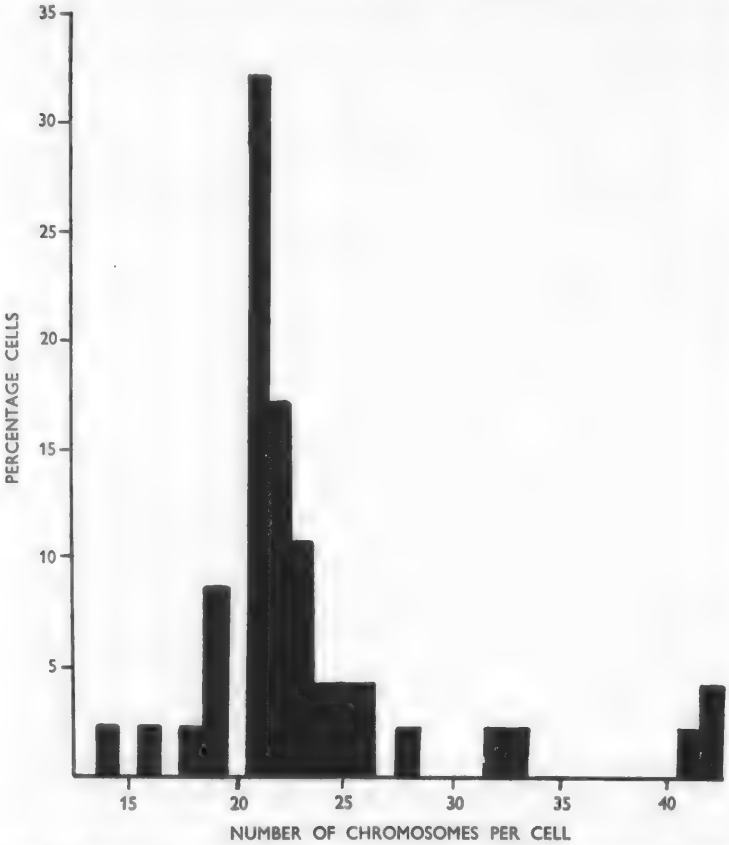

FIG. 1.—Frequency distributions of different chromosome complements per cell in root tips of the hybrid *P. typhoides* × *P. dubium* after treatment with colchicine.
A. First sixteen days after treatment.

pollen grains with only 14 chromosomes. The aneuploidy in this species, which has a most frequent chromosome number of $2n = 66$, is due to various mitotic and meiotic abnormalities. The frequency of these abnormalities varies from plant to plant and can, therefore, be assumed to be under genetic control (Gildenhuys and Brix, 1958). In its untreated hybrid with 21 chromosomes, the genes or gene complexes which control these abnormalities do not express themselves, but when the chromosome

number is doubled, even temporarily, the abnormalities again make their appearance. It would seem then that a higher gene dosage or a higher chromosome number is necessary before the abnormalities show themselves.

An explanation may lie in the relationship between the two parental species. There appears to be little affinity between *P. typhoides* and *P. dubium* (Gildenhuys, 1958), so that the hybrid is in fact a very wide cross.

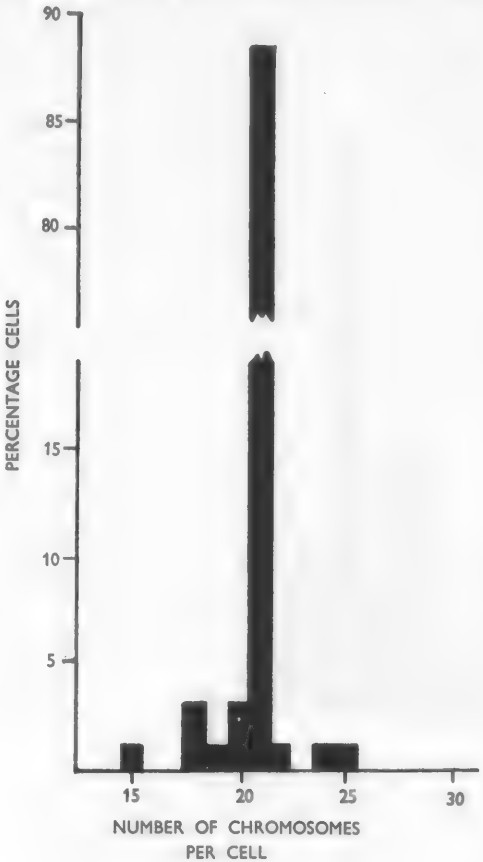

FIG. 1.
B. One year after treatment.

It is possible that a cytoplasmic barrier to the expression of genes in low dosage may occur in such a wide cross, but on doubling the chromosome number, and hence the gene dosage, this barrier is overcome and the threshold necessary for chromosome elimination is passed. Once the process of elimination becomes operative, it will continue even to the extent of producing cells with less than the original 21 chromosomes, the number of chromosomes remaining possibly depending upon which chromosomes

are eliminated. As times goes on, cells with highly reduced chromosome numbers are evidently eliminated, probably through competition, as is shown by the tendency to stabilise at 21 (cf. figs. 1A, 1B, 1C). This is probably the " balanced " number for the hybrid, in the same way as $2n = 66$ could be considered to be the " balanced " number for the polyploid parent. In the latter, which produces seed, the *status quo* is maintained by apomixis.

Whatever the mechanism which causes aneuploidy to be expressed in

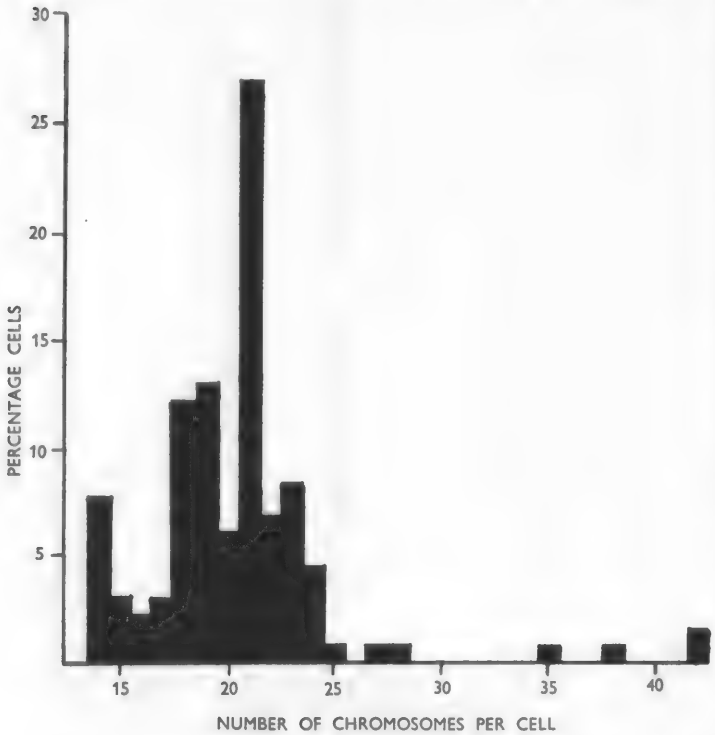

FIG. 1.

C. First sixteen days after second treatment.

the treated and not in the untreated cuttings of *P. typhoides* × *P. dubium*, the origin of the difference in response to colchicine between this hybrid and the hybrid *P. typhoides* × *P. purpureum* seems clear. The two hybrids have the same species as female parent and thus have cytoplasm and one set of chromosomes in common, so that the main difference between the two must therefore lie in the chromosomes they received from their male parents. Each received 14 chromosomes, the one from an irregular polyploid in which aneuploidy is genetically controlled, the other from a regular tetraploid (in which aneuploidy has not been reported). The aneuploidy occurring in the treated hybrid *P. typhoides* × *P. dubium* and which is absent

in treated *P. typhoides* × *P. purpureum* must therefore be assumed to be due to genes carried in the 14 chromosomes received from the polyploid parent. With these genes functioning when the chromosome number is raised, it may be impossible permanently to double the chromosome complement of such a hybrid.

5. SUMMARY

1. Two hybrids, *P. typhoides* × *P. purpureum* and *P. typhoides* × *P. dubium*, both with 21 somatic chromosomes, behave normally in mitosis.

2. When they are treated with colchicine the former produces $2n = 21$ and $2n = 42$ plants. In the latter, various mitotic abnormalities, similar to abnormalities occurring in its polyploid parent, appear and lead to chromosome numbers varying from 14 to 42, even in the same root. After one year this hybrid stabilises, through chromosome elimination and the elimination of highly reduced cells, at more or less $2n = 21$.

3. The irregular behaviour of the latter hybrid after treatment with colchicine is considered to be due to the action of genes inherited from its aneuploid *P. dubium* parent and which only express themselves when in double dosage.

4. Through genic control it may therefore be impossible permanently to double the somatic chromosome complement of *P. typhoides* × *P. dubium*.

Acknowledgment.—We are indebted to Dr A. A. Moffett for his suggestions on improving the text.

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REVIEWS

SCIENCE AND LIBERAL EDUCATION. By Bentley Glass. Louisiana State University Press, Baton Rouge. 1959. Pp. 115. $3.00.

This book, based upon a series of three lectures given at Tulane University in 1958, deals with the role of science in a modern civilisation and the need for a greater knowledge of science in a liberal education. Since the author is a geneticist it is only natural that he stresses the role of genetics in human affairs. The introductory chapter on genetics in the service of man includes some of the major contributions of genetics to agriculture and modern medicine. The value of ionising radiation to produce desired mutations in bacteria and plants is discussed, but the genetic hazards to man are also considered. Modern medicine has greatly increased the life span of modern man, but it too has created problems by preserving certain deleterious genes in the human population.

In dealing with a liberal education in the age of science the author joins C. D. Darlington (*The Conflict of Science and Society*), C. P. Snow (*The Two Cultures*) and other eminent scientists in an appeal for a better understanding of the functions of science. Science has enabled man to acquire enormous amounts of inanimate energy. This acquisition of energy leads to enormous power, which can be used for either good or evil. It can be used for man's benefit or it can be used to enslave or destroy the human race. Thus it is necessary to effect a proper balance between the sciences and the humanities to insure the wise use of science.

In the final chapter the author deals with evolution and human values, and the role of science in freeing the human race from poverty, ignorance and superstition. Early in human evolution some degree of co-operation became of value in survival. Co-operation and ethical values are even more essential for the survival of modern man because of the vast power he has acquired through the application of science to material progress. The author has dealt with these problems in a concise and effective manner.

KARL SAX.

PRACTICAL HEREDITY WITH DROSOPHILA. By Gordon Haskell. Edinburgh and London: Oliver and Boyd. 1961. Pp. xii+124. 10s. 6d.

This book deals in an introductory way with the use of *Drosophila* as a tool for illustrating the principles of genetics. It is intended for the use of schools and colleges, and includes information on the apparatus required, the techniques used in handling and feeding the flies. In addition the author has compiled a list of practical exercises together with details of mutant stocks suitable for carrying them out : the introduction includes a list of places where these stocks may be obtained. A special chapter deals with cytological procedures.

Bearing in mind that this book will be used widely in schools, issue may be taken with some of the apparatus and handling techniques which are described. A full explanation of a bottle washing machine is given. A hair-grip bent at right angles and attached to a pencil is recommended for separating the flies ; and forceps for picking them up by their wings.

But does not a camel-hair brush do both with fewer fatalities? A watch-glass fitted with a gauze pad stuck on by strips of adhesive plaster and well ethered is recommended for re-anaesthetising the flies should they wake up before the examination is completed. But would not a Petri-dish top be more suitable? And are not most types of plaster easily unstuck in the presence of ether? It is stated that " etherised female flies are reputed not to lay eggs for 48 hours afterwards " (p. 18), yet later (p. 32) the author recommends that flies should be transferred to a fresh tube two days after mating to avoid overcrowding of the resulting progeny.

Again, it is well known that better preparations of both salivary gland and ganglion chromosomes are obtained if the larvae are dissected directly in a drop of stain on a slide rather than in saline first as described (p. 108). This direct method has the added advantage of recognising the glands more easily since the large nuclei can be seen in the cells.

Most of the tricks of the trade are mentioned, and these should prove invaluable for beginners. Yet, one simple but important point is omitted—that of not allowing anaesthetised flies to fall directly onto the food in a culture bottle. It is surprising the number of students who fail to realise that flies stuck to the food will not mate or lay fertile eggs!

The mutant stocks recommended for the exercises have been well selected, and the ground covered by them is comprehensive. It is refreshing to see that exercises demonstrating polygenic inheritance together with the statistical procedures necessary for evaluating the results are included. But why place such exercises in a different chapter so giving the impression that this type of inheritance is exceptional? It is a pity that an exercise dealing directly with selection is not included.

Although this is meant to be a book dealing essentially with practical methods, one feels that the principles demonstrated by each experiment could have been more forcibly brought out. For example, in the cross of brown × scarlet-eyed flies which give a proportion of white-eyed flies in the F_2 (*bw*/*bw*, *st*/*st*) no mention was made that this illustrates that the same phenotype can be given by different genotypes (cf. the sex-linked white-eyed gene). A list of the principles demonstrated by the results of each cross, even at the risk of repetition, would have been well worth while.

Probability is a difficult concept for many beginning students to grasp. The presentation of statistical methods is admirable, but the author does not attempt to convey in simple terms the meaning of the answer derived by their application. For example, what is " p " the probability of when derived by the chi-square method? It would have been so simple to state this together with the reminder that the element of chance operates at both meiosis and fertilisation.

These defects do not distract us from recognising the usefulness of the book. There can be little doubt that its contents will be fully appreciated by teachers and students alike. E. A. BEVAN.

DARWINISM AND THE STUDY OF SOCIETY. Ed. M. Banton. Tavistock, London: Quadrangle, Chicago. 1961. Pp. 191. 21s.

A group of historians, social scientists and biologists are here introduced to us by Dr Bronowski as discussing the connection between Darwinism and the study of society. Three show us that, at least in Darwin's opinion,

there was such a connection. Eight others, however, agree that there is, or ought to be, very little connection today, save perhaps for an interesting analogy. The connection between biology and the social sciences has, they tell us, fortunately lapsed.

Is this true? Partly no doubt it is true. But it is a matter less for congratulation than for investigation. Social scientists reject the biological notions about man of Darwin and Galton together with the genetic interpretation adopted by their immediate followers like Karl Pearson. But they do not ask themselves whether a different interpretation would follow today on the basis of a somewhat more sophisticated genetics. To judge from the articles in this symposium the reason is that the modern social scientist and biologist have taken this course because separation is easier than combination. The result is historically and scientifically interesting.

The result has been a succession of misunderstandings. Stark's account of the tragedy of Gumplowicz gives a simple example. Gumplowicz, he tells us, believed that society evolved by the conflict between social classes. Upper and lower classes in England still carried on the conflict between Normans and Saxons. This, Stark says—and it would have interested Darwin to know—is "good Darwinism". Slowly Gumplowicz realised that this childish notion was not the whole truth and he abandoned it. So this was the end, or one of the ends, of Social Darwinism.

The possibility that Darwinian notions might have been indefinable in the beginning, and in process of developing in different ways ever since the beginning; or that the population genetics of class structure might have some human characteristics; that natural selection, as Galton and Pearson thought, might imply co-operation as well as conflict; or that qualitative rather than quantitative differences of ability between races and classes might govern their relations; such possibilities do not seriously trouble the contributors to this symposium. Indeed the title of the symposium itself somewhat excludes them. And in his introduction, where he skates over the surface of history wittily but not wisely, Dr Bronowski hardly introduces any misgivings.

Referring to pages 45, 52 and 87, when will British historians of Darwinism agree on how to spell Weismann's name?

C. D. D.

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